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ROGER DUHAMEL, F.R.S.C.
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A New Species of *Calocaris* (Crustacea: Decapoda, Thalassinidea) from the Northwest Atlantic[1]

By H. J. Squires

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ABSTRACT

A new species of the decapod crustacean family Axiidae, *Calocaris templemani*, from the northwest Atlantic, is described and compared with the closely related species, *Calocaris macandreae*, from the east Atlantic.

INTRODUCTION

Whiteaves (1901) reported the capture of *Calocaris macandreae* from the Gulf of St. Lawrence. This species had been named by Bell (1853) from specimens obtained off the west coast of Britain. However, the larvae are scarcely if at all planktonic (Bull, 1933) and may be expected to effect migration of the species only over a short distance. These larvae, in fact, soon settle on soft mud bottom where they form burrows in which they live for the rest of their lives (Buchanan, 1963). Also, since *C. macandreae* has never been reported from Greenland, the validity of Whiteaves' report is in question. The specimen taken by Whiteaves is no longer in existence (Dr E. L. Bousfield, National Museum of Canada, personal communication) but it must have been a different species. From all reports, it appears that *C. macandreae* is not found any farther west than southern Iceland (Wollebaek, 1908; Buchanan, 1963).

A specimen of *Calocaris* obtained from deep water in Hermitage Bay, Newfoundland, was compared with specimens of *C. macandreae* from off the coast of Northumberland, England. It was found that the specimen from Hermitage Bay differed in many particulars from *C. macandreae*. Later through the courtesy of Dr R. L. Wigley of the United States Fish and Wildlife Service, Woods Hole, Massachussetts, five specimens were obtained from the Gulf of Maine and it was found that these specimens also differed from *C. macandreae* and were similar to the specimen from Hermitage Bay.

DESCRIPTION OF THE SPECIES

Superfamily: THALASSINOIDEA

Tribe: THALASSINIDEA

Family: Axiidae

[1]Received for publication February 14, 1964.

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Calocaris templemani new species

" *macandreae* Bell 1853, Whiteaves 1901, p. 257, no figure

DESCRIPTION: The holotype is a mature adult, hermaphroditic, and 14 mm in carapace length (44 mm in total length). It is pale pink in colour. The carapace has 2 dorsal carinae each with 3–4 spines converging to form a "V" with the rostrum on the front of the carapace and continued as 4–5 spines on each side of the rostrum (Fig. 2A), each spine pointing obliquely forward and upward, but the rostrum tending to point downward (Fig. 1). Also a mid-dorsal carina extends the full length of the carapace and continues well out on the rostrum (Fig. 2A). The sternal plate of the 4th pereiopods has a relatively open notch posteriorly (Fig. 2C*Ct*). The abdomen is rather longer than the carapace and is somewhat dorso-ventrally flattened (Fig. 1); each segment, except the 1st, has dorsally two clumps of fine setae on each side of the mid line and a pleural fringe of long fine setae (Fig. 2B). The telson and uropods are short and wide and have few small carinal and lateral spines and no terminal spine on the telson (Fig. 2D). The eyes are without dark pigment and are flattened obliquely outside. The 1st antennae are about half the length of the second antennae which are about equal to the total length of the carapace+abdomen. The first section of the peduncle of the second antenna has an expansion inside with 4 short spine-like projections (Fig. 3*Ct*). The mouth parts and chelae of the pereiopods are profusely setose. The first two pairs of pereiopods are chelate. The chelae of the first pereiopods are comparatively large and carinate on dactyl and propodus (Fig. 5 *Ct*). Further details of the morphology of mouth parts, etc., are included below.

LOCALITY OF TYPE. The holotype was taken on August 13, 1960, by the C.G.S. *Marinus* in Hermitage Bay, Newfoundland, at a depth of 260 m with an otter trawl with lined cod end (temperature 4.9°C; bottom, soft mud). Collectors were E.I.S. Rees and G.E. Tucker.

REFERENCE NUMBER ALLOTTED TO TYPE. The holotype is deposited in the National Museum of Canada with the assigned Catalogue No. 5757.

LABEL ASSOCIATED WITH THE TYPE. The specimen is preserved in formalin and bears the name *Calocaris templemani* and the documentation of place, time, etc., given in the preceding paragraphs.

FIGURE OF THE TYPE. The photograph (Fig. 1) is of a specimen similar in size and morphology to the Type but from the Gulf of Maine. [Fig. 2–6 include drawings of parts of the holotype.]

EXTERNAL MORPHOLOGY

ANTENNULES (1st Ant, *Ct*, Fig. 3). The antennular peduncle has 3 sections. The first has one inner spine and many plumose setae especially over the otocyst. This segment is about equal in length to the other 2 sections combined. The flagella are long and annulate and have many long setae.

ANTENNAE (2nd Ant, *Ct*, Fig. 3). The antennal peduncle consists of 4 sections. The 1st section has an inner expansion with 4 equidistant points making the curved edge serrate (only 2 points are present on this expansion in *C. macandreae*). It has also a small papillate opening proximally, above. The 2nd section is grooved longitudinally but obliquely on both sides forming an apparent division into 2 parts. Both parts have 2 spines distally above. The 3rd section has plumose setae along the inner and outer edges. The 4th section is about one-half as long as the 3rd. The flagella of the antennae are longer than those of the antennules, and they have only occasional short setae.

FIG. 1. Photograph of *Calocaris templemani*, n. sp. Magnification about ×2.5. Specimen from the Gulf of Maine (42°10′N, 69°18′W, *Albatross* station 70, December 11, 1955, meter net) on loan through the courtesy of Dr R. L. Wigley, Woods Hole, Mass. Photo by E. L. Rowe.

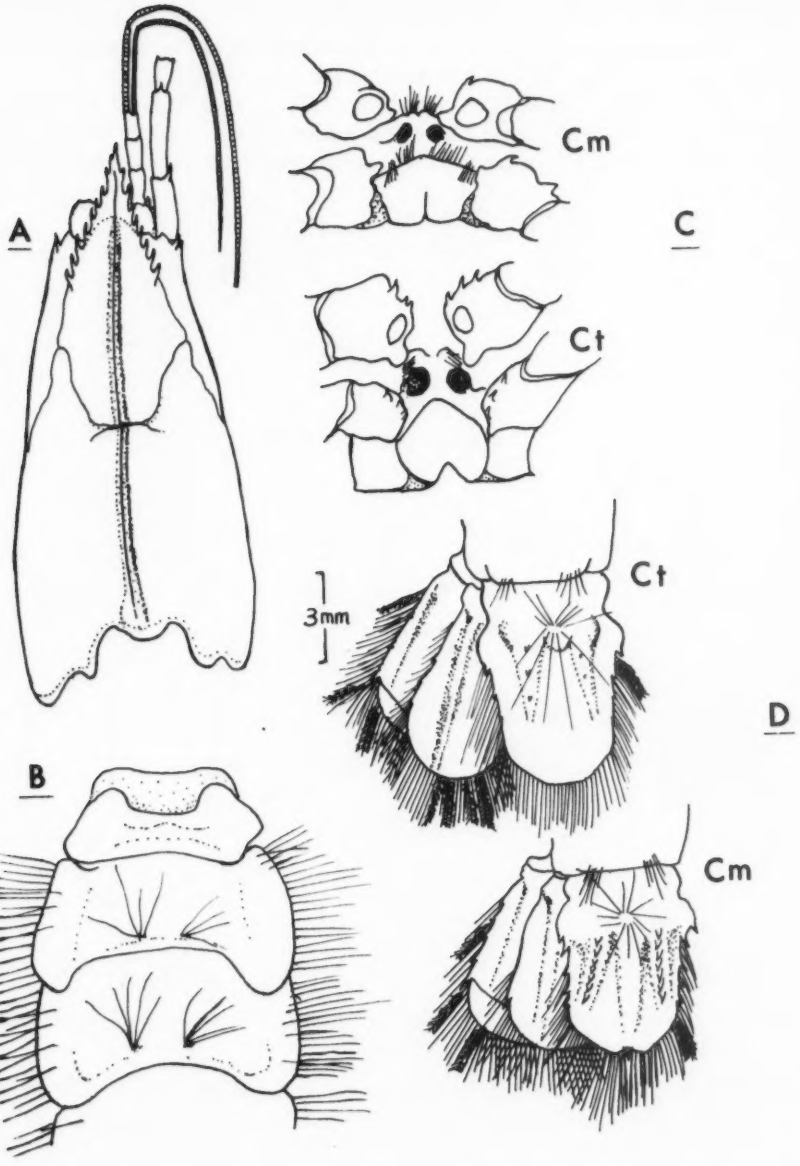

FIG. 2. *Calocaris templemani*, n. sp. (*Ct*) and *C. macandreae* (*Cm*). Carapace (A); anterior part of abdomen (B); sternal plate with ends of the 3rd and 4th pereiopods attached (C); telsons and uropods (D), carapace lengths of *Cm* and *Ct* 12 and 14 mm, respectively.

Mouth Appendages

The mouth appendages of the left side are shown in Fig. 4 and are described as follows (the legend below is as in Fig. 4):

Mandible (Mnd). The mandible is large and heavily calcified. The incisor process has an almost entire and regular edge, is slightly convex and has one small tooth at the inner end. It is separated at the inner end by a shallow

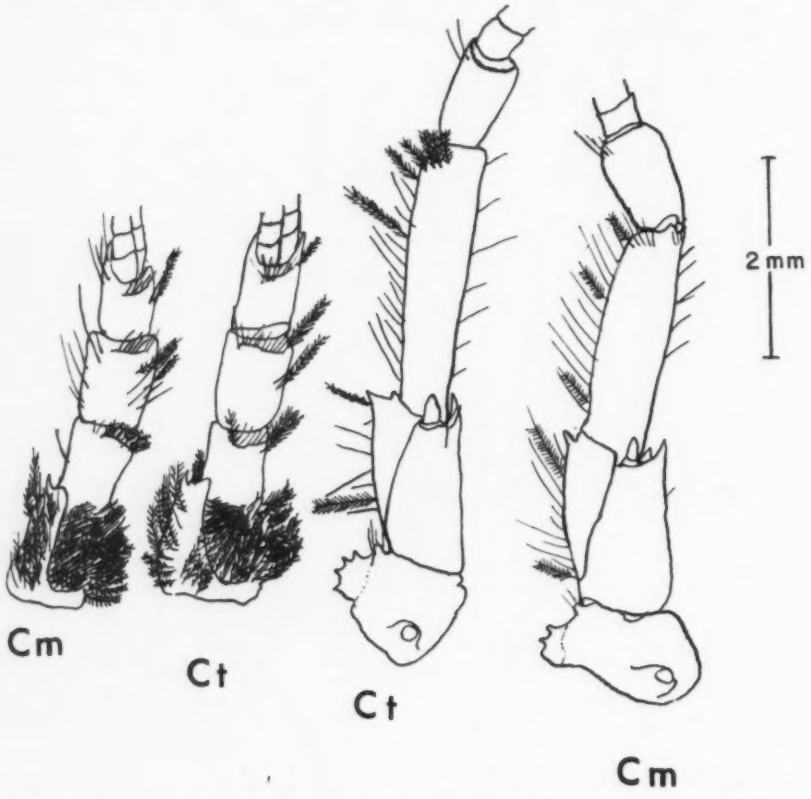

Fig. 3. *Calocaris templemani*, n. sp. (*Ct*) and *C. macandreae* (*Cm*), antennules (1st Ant) and antennae (2nd Ant).

notch from the large molar process which has a slightly hollowed elliptical surface for grinding. The palp is in 3 sections. The distal section is about equal in length to the other two combined. Half of its outer edge is fringed with bristles and there is a tuft of long bristles proximally.

First maxilla (Mx_1). The coxa is relatively large, thin, triangular in shape and lies across and outside the basis. Its inner edge is fringed by both fine and coarse setae. The basis has short stout bristles on the ventro-distal

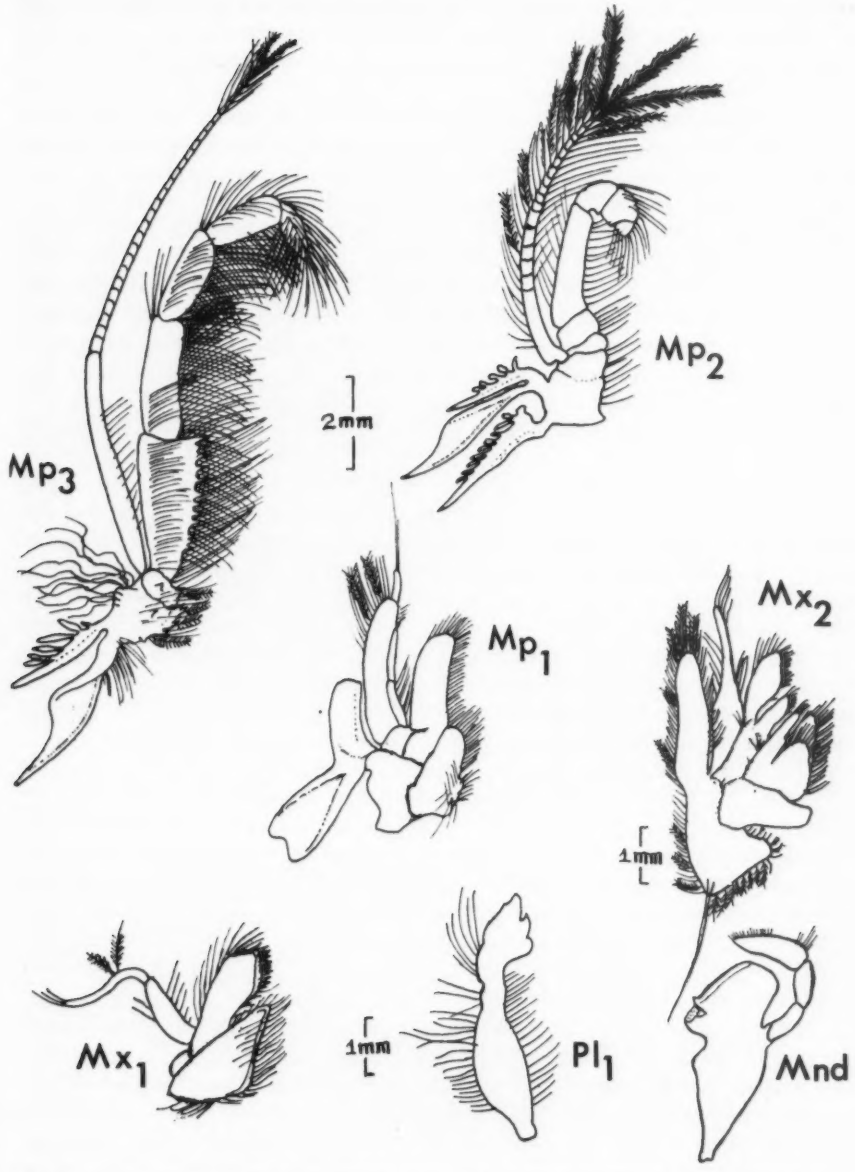

FIG. 4. *Calocaris templemani*, n. sp. mouth parts: Mp_3 = third maxilliped, Mp_2 = second maxilliped, Mp_1 = first maxilliped, Mx_2 = second maxilla, Mx_1 = first maxilla, Mnd = mandible. Also Pl_1 = first pleopod.

edge and fringes of setae laterally. The endopod is in 2 sections, the proximal of which has a few setae at its inner edge. The distal section is recurved and flagella-like. It has 2 prominent plumose setae at the point of greatest flexure and distally 3 simple setae.

SECOND MAXILLA (Mx_2). The precoxa is short and wide. The coxa, which is bilobed, has a triple fringe of setae on the large proximal lobe: the central fringe is on the pointed edge of the lobe and the other two originate at about one-third the length of the lobe from its base and form an arc toward its apex on both the outside and inside of the lobe. The smaller lobe has a setal fringe which is longest at the tip of the lobe. There is a setal fringe also on the edges of both lobes of the basis. The endopod which is attached to the basis and coxa has a few long setae most of them distal and on the inner edge. The exopod is attached to the basis and precoxa. It has a fringe of plumose setae, the longest of which are distal. It has a triangular extension proximally, the scaphognathite, which also has a fringe of plumose setae. In some specimens this proximal extension has a long apical seta as in *C. macandreae*, but in others the seta is lacking.

FIRST MAXILLIPED (Mp_1). The precoxa is short and wide with the exopod and endopod attached distally, an epipod attached laterally outside, and the coxa attached laterally inside. The coxa and basis both have an inner fringe of setae. The coxa is small and supports the basis. The endopod is slender and is divided into 2 sections which are equal in length and the proximal of which has a few setae. The exopod is longer than the endopod and has a finger-like projection at the tip with 2 long apical setae. It also has a short fringe of long plumose setae outside and distally, while on the inside there is a fringe of simple setae. The epipod is bilobed and the posterior extension, which has skeletal support, acts as a scaphognathite. The anterior extension is rounded.

SECOND MAXILLIPED (Mp_2). The coxa is large and somewhat irregular in shape. The basis is short, wedge-shaped, and crowded under the ischium which is approximately one-quarter the length of the merus. The carpus forms the permanent ventral flexure of the endopod. The propodus is flat and wide, and the dactyl small, flat, and triangular in outline. The endopod has setal fringes especially apically where some are spine-like. The exopod is attached to the coxa. The distal three-quarters of its length is annulated (about 15 annulations) and many lateral and terminal plumose setae are present. Also attached to the coxa are a triangular-shaped epipod and 2 podobranchs.

THIRD MAXILLIPED (Mp_3). The endopod is very much longer than that of the second maxilliped. It has profuse long setae especially along the inner edge and terminally where some of them are spine-like. The coxa and basis are closely coalesced and bear the exopod, epipod, and one podobranch. Each of the coxa and basis bears a short stout spine on the inner side and short plumose setae. Outside, the large triangular-shaped epipod has skeletal stiffening and an arcuate base with long setae opposite to where the podobranch is joined to it. The ischium of the endopod is triangular in cross-section and has 12 strong pointed teeth on the inner edge forming a gnathopod. The merus is about as long as the

ischium, and the carpus and propodus are slightly shorter than the merus but about equal in length. The dactyl is short and conical in shape. The exopod has an annulated flagellum which is about one and one-half times the length of its basal section. It has about 7 pairs of plumose setae distally.

Other Appendages

First pereiopod. (The chelae of the left 1st pereiopods of *C. templemani* (*Ct*) and *C. macandreae* (*Cm*) are shown in Fig. 5.) The chela of *C. templemani* may be described as follows: the edges of the dactyl and propodus are fringed with profuse long setae. On each side of the dactyl is a ridge which has a fringe of long setae for its whole length. The teeth of the shearing edge of the dactyl

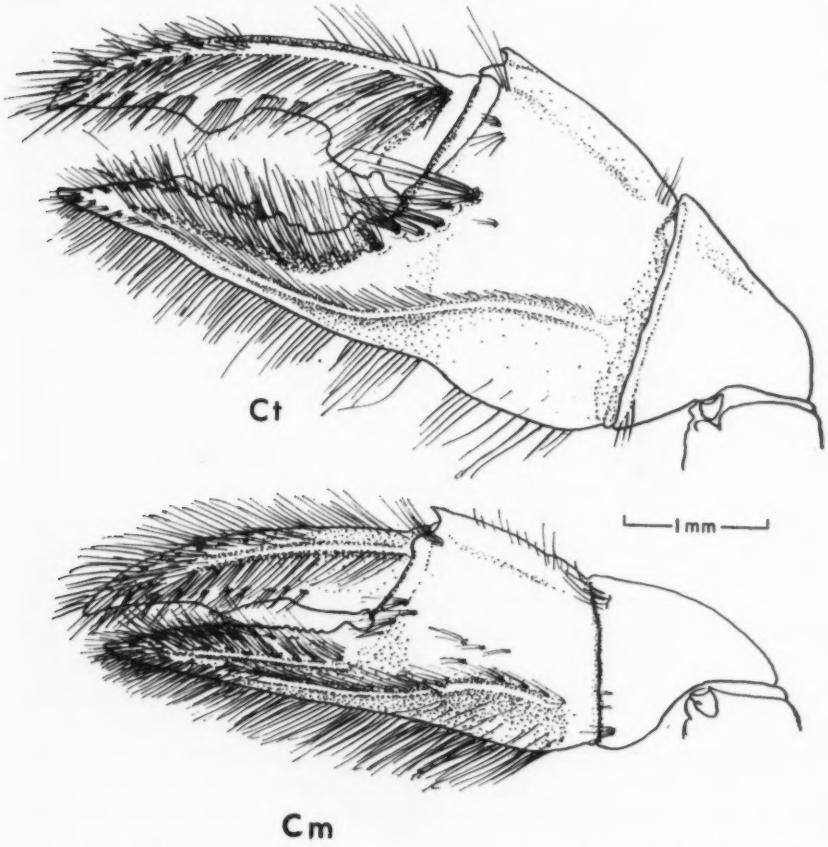

Fig. 5. *Calocaris templemani*, n. sp. (*Ct*) and *C. macandreae* (*Cm*), left chelae of first pereiopods (both chelae of the first pereiopods in the same species are essentially similar but may be slightly unequal in size in some).

are large and 2 in number with a large gap between them. The shearing edge of the propodus has about 7 teeth varying in size, and the most proximal of these is more prominent than the others. Prominent ridges at the inferior edge of the propodus on the outside and inside give a somewhat oblique flattened lower edge to the chela. The outer ridge has a conspicuous spine at one-third of its length proximally in *C. macandreae*. This is lacking in *C. templemani*. At the superior short edge of the propodus (the top of the chela) is a prominent ridge terminating distally in a spine.

SECOND PEREIOPOD. This is much smaller than the 1st pereiopod. It is chelate and very setose (Fig. 6).

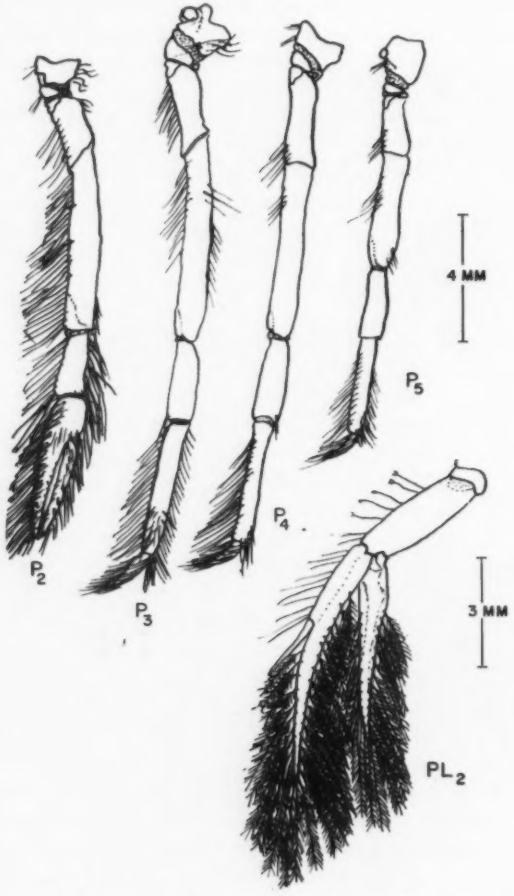

FIG. 6. Pereiopods ($P_2 - P_5$) and pleopod (Pl_2) of *Calocaris templemani*, n. sp.

THIRD TO FIFTH PEREIOPODS. These are not chelate. The 3rd and 4th are about equal in size, but the 5th is considerably shorter, mostly because of a short ischium (Fig. 6). The 2nd to 5th pereiopods of *C. templemani* do not differ in any outstanding feature from the corresponding pereiopods of *C. macandreae*.

FIRST PLEOPOD (Pl_1). The 1st pleopod is different from the others. It is undivided and somewhat spatulate with a light fringe of setae and a curved foliaceous apex (Fig. 4).

SECOND TO FIFTH PLEOPODS (PL. Fig. 6). These are the same as in other decapods but have a short coxa and long basis and 2 rami, an exopod and an endopod. The endopod has a long narrow appendix interna. Both exopod and endopod are fringed by long plumose setae. There are "brush" setae present, as described by Buchanan (1963) for *C. macandreae*. They are found at the inner edge and distally on the basis for the most part.

The pleura of abdominal segments 2–6 are fringed ventrally with long simple setae (a few present near the edge of each pleuron especially on segment 5). Shorter plumose setae extend inward from the ventral edge of the pleura. Dorsally, a tuft of long simple setae is present on each side of the mid line on abdominal segments 2–5 (Fig. 2).

DISCUSSION

DISTRIBUTION. *Calocaris templemani* appears to be found only in the west Atlantic, and its closely allied species, *C. macandreae*, appears to be confined to the east Atlantic. Four other species of decapods also limited in their northward distribution by the Strait of Belle Isle are confined to the west Atlantic, but there are species closely resembling them which are confined to the east Atlantic and are not found in Greenland (Allen and Squires, in preparation). The 4 pairs of species are as follows (those from the east Atlantic are in parentheses): *Crangon septemspinosus* (*C. crangon*), *Dichelopandalus leptocerus* (*D. bonnieri*), *Homarus americanus* (*H. gammarus*) and *Pagurus acadianus* (*P. bernhardus*). In *Calocaris* also the separation in distribution of the east and west Atlantic species appears to have resulted in significant differences in morphology.

COMPARISON OF *C. templemani* (*Ct*) WITH *C. macandreae* (*Cm*). Both species (see Table I for similarities and differences in morphology) look rather alike superficially but the outstanding differences are:

(a) *Ct* has a larger number of spines than *Cm* on the rostrum and anterior part of the carapace.

(b) The mid-dorsal carina extends out on the rostrum in *Ct* while in *Cm* this carina ends at the anterior edge of the carapace.

(c) The basal section of the 2nd antenna of *Ct* has an inner expansion with 4 projections while *Cm* has but 2 projections.

(d) The sternal plate of the 4th pereiopods in *Ct* has an open notch posteriorly. This is closed in *Cm*.

(e) The lateral edges and dorsal carinae of the telson in *Ct* have fewer and weaker spines than in *Cm*. The posterior end of the telson is truncate but

TABLE I. Comparison of *Calocaris macandreae* from off the coast of Northumberland, England, with *C. templemani* from Hermitage Bay, Newfoundland (Fig. 2–5). Counts of spines were obtained from 14 specimens of *C. macandreae* and 5 specimens of *C. templemani*.

Calocaris macandreae	*Calocaris templemani*
1. Rostrum with point turning upward and with one spine at each side proximally plus 3 on each rostral-carapacial carina	1. Rostrum with point directed downward, about 5 spines on the left and right side of rostrum plus 3 on each rostral-carapacial carina.
2. Mid-dorsal carina begins on the carapace anteriorly and extends the full length of the carapace with one interruption at the cephalo-thoracic groove.	2. Mid-dorsal carina begins well out on the rostrum anteriorly and extends the full length of the carapace with one interruption at the cephalo-thoracic groove.
3. Rudimentary eye with a flat oblique surface facing sideways and forward, with the anterior edge rounded.	3. Rudimentary eye with a flat oblique surface facing sideways and forward but with the anterior edge almost rectilinear.
4. Antennal spine on the carapace small and directed forward.	4. Antennal spine on carapace large and directed forward and outward.
5. Telson rounded posteriorly but with a shallow cleft in which there is a small spine.	5. Telson rounded posteriorly and with a very shallow depression but without a spine.
6. Telson carinae have 3–7 strong spines each, all directed posteriorly.	6. Telson carinae have 0–3 very small low spinules each.
7. Proximally the edge of the telson has a lateral expansion and usually 2 strong spines directed posteriorly on each side.	7. Proximally the edge of the telson has a lateral expansion with 1 spine only directed posteriorly on each side.
8. The 4th sternite has a narrow groove posteriorly which extends to a little more than half the length of the sternal plate.	8. The 4th sternite has a wide notch posteriorly and extending into the sternal plate about one-third of its length.
9. The shearing edge of the dactyl of the 1st pereiopod has one low tooth and the propodus has about 14 small even teeth.	9. The shearing edge of the dactyl of the 1st pereiopod has 2 large teeth, and the propodus has about 7 teeth varying in size.
10. On the 1st segment of the antenna an inner expansion has but 2 spines at its edge (Fig. 5).	10. On the 1st segment of the antenna an inner expansion has a serrate edge of 4 spines (Fig. 5).

has no spine in *Ct* while in *Cm* this end is slightly notched and there is a small distinct spine.

(f) The teeth of the chelae of the 1st pereiopod of *Ct* are larger and fewer in number than those of *Cm*. A spine is present on the outside carina of the propodus in *Cm* but is lacking on this carina in *Ct*.

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I wish to express my thanks to Dr J. B. Buchanan of the Dove Marine Laboratory for many discussions about *Calocaris macandreae* and for making available specimens of this species for comparison with *C. templemani*. A special word of thanks is due to Dr R. L. Wigley of the United States Fish and Wildlife Service, Woods Hole, Mass., for the loan of specimens taken in the Gulf of Maine.

This paper was written and the work on which it is based was done at the Dove Marine Laboratory, Cullercoats. The space provided and the services rendered by the Deputy Director and staff are sincerely appreciated.

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Effects of Salting and Smoking on Protein Quality of Cod[1]

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ABSTRACT

Salting and smoking had no effect on the biological value of cod protein, as indicated by Protein Efficiency Ratio, gross protein values, total lysine and methionine content, "available lysine" values, and plasma free lysine and methionine levels in human subjects. It was concluded that smoked and salted codfish can provide protein of high nutritional value.

INTRODUCTION

Although salted and smoked cod have been used as foodstuffs for many years, surprisingly little is known of their nutritive value. In a recent review article, Cutting (1961) noted that salting may slightly decrease the total nitrogen content of fish, presumably by leaching out of proteinaceous material. As one might expect, this effect is more pronounced in heavily salted fish than in those treated with a mild salt cure. Little is known of possible effects of mild smoke curing on the nutritional value of fish protein although data presented by Orraca-Titteh (1961) indicate apparently reduced net protein value in smoked products.

This report deals with studies on the effects of salting and smoking on the nutritional value of the protein in cod (*Gadus morhua*).

MATERIALS AMD METHODS

Medium salted (moisture 48%, salt 20%), cold smoked, and fresh cod fillets were obtained on the local market and cooked in boiling water using household methods of preparation. The material then was freeze-dried, ground to 20-mesh and analysed.

Analytical Procedures

The samples were analyzed for moisture and total nitrogen by methods given by the AOAC (1960). "Available lysine" was determined by the method of Carpenter (1960) and total methionine was estimated by the chemical procedure of McCarthy and Paille (1959). Total lysine and methionine was determined microbiologically by the procedure of Steele *et al.* (1949), using *L. mesenteroides* P-60 as the test organism.

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Animal Studies

Male weanling (23–25 days) Wistar strain rats of the Food and Drug colony were housed individually in screen bottom cages kept in an air-conditioned room maintained at 23–24°C and given food and water *ad libitum*. Protein Efficiency Ratio (PER) values were determined by the method of Chapman *et al.* (1959). Gross protein values were determined by the method of Morrison and Sabry (1963). A group of 10 rats received a basal methionine-deficient diet containing 8% protein from cooked peas. Comparable groups were given the basal diet supplemented with 2% protein from casein, fresh fish, smoked fish, or salted fish. Gross protein values were calculated after 2 weeks.

Human Bioassay Studies

Nine members of the laboratory staff were divided into two groups. After fasting for 12 hr the members of one group received a test meal of 20 g of protein from salted fish while the other group received a similar amount of protein from fresh fish. Blood was taken by finger prick at the start of the meal and at 1 and 3 hr after consumption. The plasma was analyzed for lysine and methionine by the method of McLaughlan *et al.* (1961). In a second assay the groups were reversed so each individual served as his own control. In other studies the procedure was repeated with six subjects, except that smoked fish was substituted for salted fish. Results were expressed as the mean of the value at 1 and 3 hr after the meal minus the fasting value.

RESULTS AND DISCUSSION

The results presented in Table I indicate that the smoked and salted fish contained amounts of protein, lysine, and methionine similar to those in the fresh sample. Methionine values determined chemically were somewhat higher than those obtained by the microbiological procedure. "Available lysine" values also were similar in fresh, smoked, and salted material. Values for total lysine were considerably greater than those for "available lysine". It is possible this large difference may be related, in part, to the presence of

Table I. Effect of salting and smoking on total and available lysine and methionine in cod.

Sample	Moisture (%)	Protein (%) (N×6.25)	Available lysine (g/16 g N)	Total lysine (g/16 g N)	Total methionine (g/16 g N)	
					Micro-biological	Chemical
Fresh fish	11.8	78.1	7.9	12.3	3.7	3.9
Smoked fish	11.9	77.0	7.9	12.8	3.7	4.3
Salted fish	11.8	78.0	7.9	11.7	3.5	3.9

hydroxylysine in the samples. It is known from the work of Sauberlich (1957) and Carpenter (personal communication) that hydroxylysine will stimulate the growth of *L. mesenteroides* P-60 at low levels of lysine. This synergistic effect results in error in lysine determination.

Biological data on the nutritive value of the three samples of fish are summarized in Table II. PER and gross protein values were significantly greater for fresh, smoked, or salted fish than for casein. No significant differences were observed between the samples of fish. Both PER and gross protein value determinations reflect the amount and availability of the limiting amino acid in the diet (Morrison and Sabry, 1963). It is evident, therefore, that smoking and salting had no effect on the availability of the sulfur amino acids, which are limiting in fish protein for the growing rat.

TABLE II. Effect of salting and smoking on nutritive value of protein in cod.

Protein	Food intake (g/4 weeks)	Weight gain (g/4 weeks)	PER (g gain/g protein)	Gross protein value
Casein	246±8.5[a]	73.4±4.5	2.98±1.06	6.77±0.62
Fresh fish	284±3.7	101.0±2.1	3.56±0.08	7.42±0.47
Smoked fish	264±7.8	95.8±4.0	3.63±0.07	7.68±0.72
Salted fish	289±8.4	106.0±1.6	3.67±0.04	7.58±0.48

[a]Standard error.

Further evidence that smoking and salting did not effect availability of amino acid is summarized in Table III. Plasma free lysine and methionine values in human subjects given the processed fish samples were similar to those found after giving fresh fish.

TABLE III. Plasma free lysine and methionine values in human subjects given fresh, salted, or smoked cod.

Test	Product	Lysine (μg/ml)		Methionine (μg/ml)	
		Fasting level	Mean increase[a]	Fasting level	Mean increase[a]
1	Fresh	25.1	31.7	3.33	4.90
	Salted	24.8	32.4	3.41	5.10
2	Fresh	34.2	37.8	3.38	4.51
	Smoked	34.3	40.0	3.41	4.50

[a]Results expressed as mean of values at 1 and 3 hr minus the fasting level.

Although it may contribute significant amounts of other nutrients to the diet, the primary nutritional contribution of fish is high-quality protein. Increased use of fish is indicated, therefore, in developing areas of the world in which protein-calorie malnutrition is widespread, particularly in pre-school children. However, economic and other factors often limit the use of fish in fresh, frozen, canned, or dehydrated forms in technologically underdeveloped countries. The present studies suggest that more traditional methods of preserving fish can be of importance in assuring products of high nutritional value.

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Carbonyl Compounds in Salted Cod. I. Thin-Layer Chromatography of Aliphatic Monocarbonyl 2,4-Dinitrophenylhydrazones[1]

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ABSTRACT

Methods are described for the separation of 2,4-dinitrophenylhydrazones of normal aliphatic monocarbonyl compounds by thin-layer chromatography. Magnesia-cellulose thin-layer plates are used to separate mixtures of these compounds into classes (*n*-alkanals, *n*-alkan-2-ones, *n*-alk-2-enals, and *n*-alka-2,4-dienal). Propylene glycol- and petrolatum-impregnated silica gel plates were also used to separate each class into homologues. The former impregnant resolved the compounds with less than seven carbons and the latter with more than six carbons. Two-dimensional separation was accomplished by resolving a mixture of these compounds into classes on magnesia-cellulose in the first dimension and the classes into homologues on either petrolatum- or propylene glycol-impregnated silica gel plates in the second dimension.

INTRODUCTION

It has been recognized that carbonyl compounds are associated with the flavours of food products. In the characterization of these compounds the investigators usually first isolated the carbonyls and then converted them into 2,4-dinitrophenylhydrazones. These derivatives were then separated and identified by chromatography. In the separation of these compounds thin-layer chromatography has not been used as extensively as other chromatographic methods.

Thin-layer chromatography, to some extent, was used to separate the mixtures of normal aliphatic monocarbonyl 2,4-dinitrophenylhydrazones according to classes. Badings (1959) employed starch-bound zinc carbonate thin-layer plates to separate deca-2,4-dienal and oct-2-enal with light petroleum-benzene (9:1) containing some pyridine. This method was adapted from van Duin's method (1958) which uses a zinc carbonate column to separate a 2,4-dinitrophenylhydrazone mixture of classes into *n*-alkanals, *n*-alkan-2-ones, *n*-alk-2-enals, and *n*-alka-2,4-dienals. Later, Urbach (1963) on aluminum oxide G and 4% diethyl ether in light petroleum (b.p. 30–40°) separated a mixture of *n*-alkanal, *n*-alkan-2-one, and *n*-alk-1-en-3-one 2,4-dinitrophenylhydrazones according to classes. She also separated a class mixture of *n*-alkanal, *n*-alk-2-enal, and *n*-alka-2,4-dienal 2,4-dinitrophenylhydrazones on plates made from aluminum oxide G containing 25% silver nitrate and 16% diethyl ether in light petroleum (b.p. 30–40°). This system also separates derivatives of the isomers, *n*-nona-*trans*-2,*trans*-6-dienal, and *n*-nona-*trans*-2,*cis*-6-dienal.

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Individual class mixtures of monocarbonyl 2,4-dinitrophenylhydrazones were separated into homologous members by thin-layer chromatography. Onoe (1952) used silica gel plates and water-saturated benzene to separate citral, *n*-alkanals C_{1-10}, propenal, and but-2-enal 2,4-dinitrophenylhydrazones. The 2,4-dinitrophenylhydrazones of some aromatic and normal aliphatic aldehydes were separated with 5% ethyl acetate in benzene and 3:1 mixture of benzene in light petroleum (b.p. 60–70°), respectively, on silica gel G plates (Dhont and deRooy, 1961). The 2,4-dinitrophenylhydrazones of some carbonyl compounds were separated on alumina with either a 1:1 mixture of hexane–benzene or diethyl ether–benzene (Rosmus and Deyl, 1961) and on neutral alumina with hexane–chloroform–phenyl nitrate (8:2:1) or 2:1 mixture of cyclohexane–phenyl nitrate (Nano and Sancin, 1963). Urbach (1963) adapted the paper chromatographic method (Lynn *et al.*, 1956) to thin-layer chromatography, where 2-phenoxyethanol-impregnated kieselguhr G plates and light petroleum (b.p. 100–120°) were used to separate all the members of the normal homologous series of alkanals C_{1-14}, alkan-2-ones C_{3-13}, alk-1-en-3-ones C_{4-10}, alk-2-enals $C_{3-11,16}$, alk-2,4-dienals $C_{5-12,14,16,18}$, and alk-3-en-2-ones $C_{6,7,10}$ as 2,4-dinitrophenylhydrazones.

This report describes the methods for the separation of a mixture of 2,4-dinitrophenylhydrazones into classes (*n*-alkan-2-ones, *n*-alkanals, *n*-alk-2-enals, and *n*-alk-2,4-dienal) and individual classes into homologous members on magnesia-cellulose plates and silica gel G plates impregnated with propylene glycol and petrolatum, respectively.

EXPERIMENTAL

Materials

Chloroform (ACS), heptane (b.p. 96–99°), and cyclohexane were purified as described by Hornstein and Crowe (1962). Absolute ethanol, 0.75% by volume, was added to purified chloroform. Absolute methanol and commercial ethanol were refluxed and distilled from sodium hydroxide (Ellis *et al.*, 1958). White petrolatum, U.S.P., was purchased from Metheson, Coleman and Bell, Rutherford, N.J., U.S.A., propylene glycol from Eastman Organic Chemicals, Rochester, N.Y., Magnesia 2665, M-302, from Fisher Scientific Co., Montreal, Quebec, silica gel G from E. Merck, AG Darmstadt, West Germany, and MN-cellulose powder from Macherey, Nagel and Co., Düren, Germany.

Hydrazone Preparation

The 2,4-dinitrophenylhydrazones of commercially available *n*-alkanals $C_{1-5,\ 7-11}$, 2-methylalkanals C_{4-6}, *n*-alkan-2-ones $C_{3-5,\ 7-9,\ 11}$, *n*-alk-2-enals $C_{3,4,6,9}$, and hexa-2,4-dienal were prepared by the method of Shriner and Fuson (1948). The derivatives were recrystallized to a constant melting point. Standard solutions were prepared containing 0.1–0.2 μg of each compound per μliter of ethanol.

Class Separation

A mixture of 2,4-dinitrophenylhydrazones was separated into classes, viz., *n*-alkanals, 2-methylalkanals, *n*-alkan-2-ones, *n*-alk-2-enals, and *n*-alka-2,4-dienals, on magnesia columns using increasing proportions of chloroform to hexane in a stepwise manner (Schwartz *et al.*, 1962). This technique was adapted to thin-layer chromatography which is now described.

Magnesia (30 g) and cellulose (5 g) were sieved through a 100-mesh screen, slurried in 90 ml of water and applied on five plates with an applicator set to 0.37 mm. The plates were dried at room temperature, activated at 110°C for 1 hr and stored over anhydrous calcium sulfate (Drierite) in a desiccator. The plates, spotted with a 10-μliter pipette, were developed in a tank, containing 140 ml of cyclohexane–chloroform (4:3), at 24–26°C, until the solvent front rose 15 cm above the origin. Development time was about 2.5 hr. Records of the chromatograms were made by tracing the spots on onion skin paper under fluorescent or ultraviolet light. Spots were scraped off and eluted with chloroform or ethanol for further separation.

Separation of Classes into Homologues

Separation of individual classes, i.e. *n*-alkanone, *n*-alkanal, *n*-alk-2-enal, and *n*-alka-2,4-dienal 2,4-dinitrophenylhydrazones, into homologous members was accomplished by paper chromatography using propylene glycol-Skellysolve C-methanol and petrolatum-aqueous methanol systems (Ellis *et al.*, 1958; Gaddis and Ellis, 1959). These systems were adapted to the following thin-layer chromatographic separations. Silica gel G (30 g), slurried in 65 ml of water, was applied 0.25 mm thick on the plates and dried overnight at room temperature. The plates were then impregnated with the propylene glycol–methanol (1:4) or petrolatum–heptane (7:100, w/v) by covering the plate with the respective impregnating solutions and draining off the excess. After application of the samples the propylene glycol-impregnated silica gel G plates were developed in a paper lined tank containing 120 ml of the heptane phase from heptane–methanol (24:1). The petrolatum-impregnated silica gel G plates were developed in a tank containing 120 ml methanol–water (9:1). Development for both systems was carried out at 24–26°C and was continued until the solvent front rose 15 cm above the origin. Development time for the former system was about 1 hr and for the latter about 2 hr.

Two-Dimensional Chromatography

A mixture of normal aliphatic monocarbonyl 2,4-dinitrophenylhydrazones was resolved in the first dimension on activated magnesia-cellulose and in the second dimension on the same plate impregnated with propylene glycol or petrolatum. The first dimension separates the classes and the second dimension separates the homologous members of classes. The samples were spotted 1.5 cm from the solvent edge and 3 cm from the other edge of the plate. After

development of the first dimension, the plates were dried and placed in a vertical position and impregnated by carefully pouring the impregnating solution from a 10-ml pipette close to the developed spots and parallel to the nearest edge. The conditions of development were the same as previously described.

RESULTS AND DISCUSSION

The separation of homologous members of individual classes [viz., 2-methylalkanals (1), *n*-alkanals (2), *n*-alkan-2-ones (3), *n*-alk-2-enals (5), and *n*-hexa-2,4-dienal (8), a mixture of classes (4), excluding 2-methylalkanals and partial mixture of classes (6 and 7)] as 2,4-dinitrophenylhydrazones on magnesia-cellulose thin-layer plates is shown in Fig. 1. The abnormal mobility of

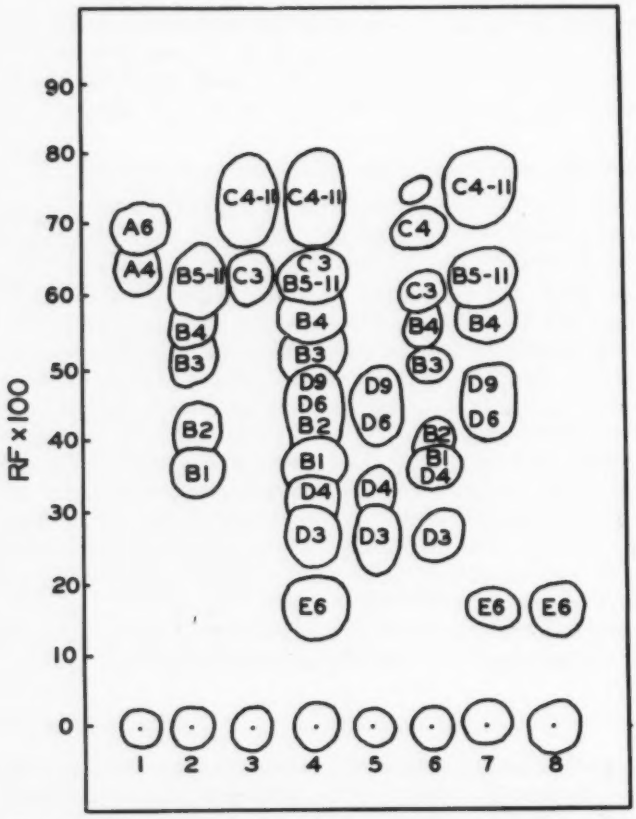

FIG. 1. Thin-layer chromatogram of normal monocarbonyl 2,4-dinitrophenylhydrazones on magnesia-cellulose. A = 2-methylalkanals; B = *n*-alkanals; C = *n*-alkan-2-ones; D = *n*-alk-2-enals; E = hexa-2,4-dienal. Numbers after the letters represent the chain length. Amount applied, 6 μliters.

formaldehyde (B1), acetaldehyde (B2), propanal (B3), acetone (C3), acrolein (D3), and but-2-enal (D4) behind their respective classes is similar to that on magnesia columns (Schwartz *et al.*, 1962). A clear-cut separation of longer chain members as classes was achieved (see spot 7). The presence of 2-methylalkanals will bridge the *n*-alkan-2-one and *n*-alkanal spots and thereby prevents their separation.

The use of cellulose with magnesia reduces the development time and improves resolution.

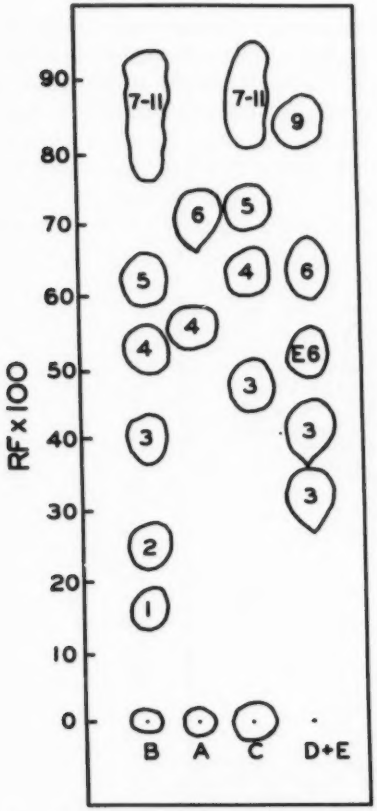

FIG. 2. Thin-layer chromatogram of monocarbonyl 2,4-dinitrophenylhydrazones on propylene glycol-impregnated silica gel G. A = 2-methylalkanals; B = *n*-alkanals; C = *n*-alkan-2-ones; D = *n*-alk-2-enals; E = hexa-2,4-dienal. The numbers represent the chain length. Amount applied, 6 μliters.

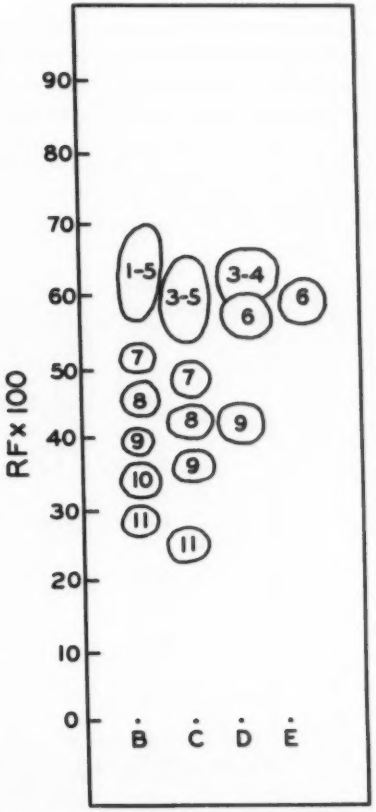

FIG. 3. Thin-layer chromatogram of monocarbonyl 2,4-dinitrophenylhydrazones on petrolatum-impregnated silica gel G. B = *n*-alkanals; C = *n*-alkan-2-ones; D = *n*-alk-2-enals; E = hexa-2,4-dienal. The numbers represent the chain lengths. Amount applied, 6 μliters.

The colour of the class spots, when the plates are wet, is somewhat the same as the respective class bands on the magnesia column, where the *n*-alkan-2-one, *n*-alkanal, *n*-alk-2-enal, and *n*-alka-2,4-dienal derivatives are gray, tan, rust-red and lavender, respectively (Schwartz *et al.*, 1962). The colour intensity of the spots decreases somewhat when the plates become dry. This characteristic colour, along with the mobility of the spots, aids in the identification of the compounds.

From the spots remaining at the origin, it appears that the monocarbonyl derivatives are absorbed or broken down on the magnesia plates, which suggests that the usefulness of this method is limited to qualitative or at best, semi-quantitative work.

The compounds of individual classes may be separated by thin-layer chromatography on propylene glycol- or petrolatum-impregnated silica gel G plates. This is shown in Fig. 2 and 3, which illustrate the separation of 2,4-dinitrophenylhydrazones of 2-methylalkanals (A), *n*-alkanals (B), *n*-alkan-2-

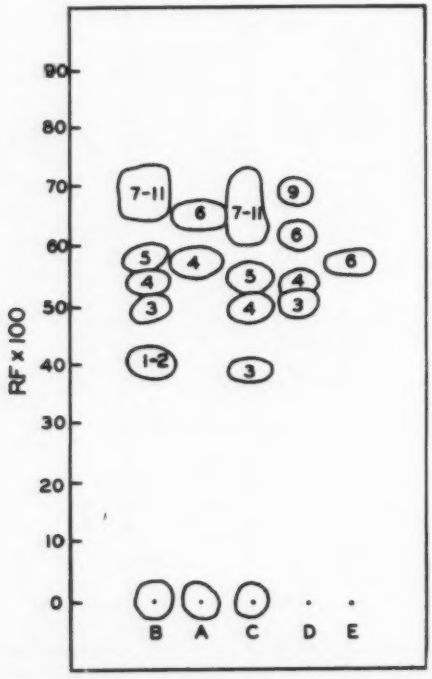

FIG. 4. Thin-layer chromatogram of monocarbonyl 2,4-dinitrophenylhydrazones on silica gel G activated at 110°C. A = 2-methylalkanals; B = *n*-alkanals; C = *n*-alkan-2-ones; D = *n*-alk-2-enals; E = *n*-hexa-2,4-dienal. The numbers represent the chain length. Amount applied, 8 μliters; development time, 70 min.

ones (C), *n*-alk-2-enals (D), and *n*-hexa-2,4-dienal (E) into their homologues. Propylene glycol resolves the class homologues with chain length of about six carbons or less, while petrolatum resolves the higher chain length compounds. The separation pattern of these two chromatograms approximates that on paper impregnated with the corresponding immobile phases (Gaddis and Ellis, 1959). The 2-methylalkanals move slightly ahead of the corresponding *n*-alkanals on propylene glycol-impregnated plates. The distance between the spots is quite constant, however, the R*f* values vary from plate to plate or on the same plate if the absorbent is uneven or the spots are too near the edges. Standards therefore must be used alongside the unknowns. Overloading of the spots will cause trailing.

Figure 4 shows the separation of individual classes on silica gel G plates, activated at 110°C for 1 hr, with Onoe's (1952) solvent system of water-saturated benzene. The same results were obtained with solvent systems of heptane–benzene (3:1), and that of Dhont and deRooy (1961), who used hexane–benzene (3:1) to separate the normal saturated aldehyde 2,4-dinitrophenylhydrazones on silica gel G plates. The above systems separate, although not clear-cut, the class members of six carbons or less, formaldehyde, acetaldehyde, and acetone can be separated as one spot. This method can be used in the

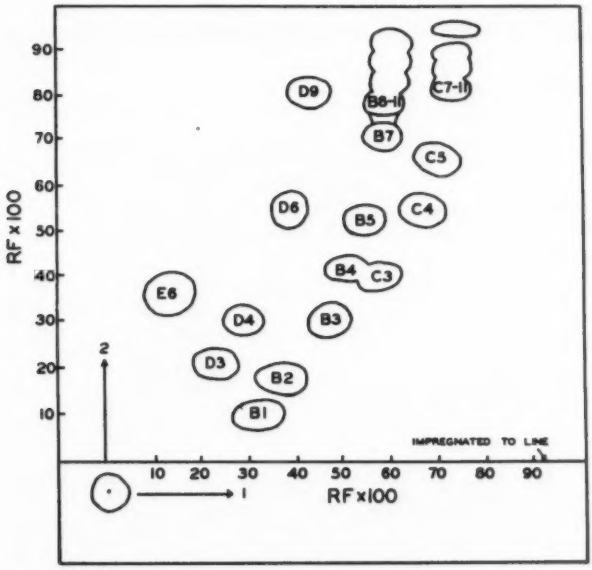

FIG. 5. Two-dimensional thin-layer chromatogram of monocarbonyl 2,4-dinitrophenylhydrazones on (1) magnesia-cellulose and (2) on same plate impregnated with propylene glycol. B = *n*-alkanals; C = *n*-alkan-2-ones; D = *n*-alk-2-enals; E = *n*-hexa-2,4-dienal. The numbers represent the chain length. Amount applied, 6 μliters.

removal of some of the anomalous members from a mixture, thus aiding the subsequent separation according to classes on magnesia-cellulose plates.

The unidimensional thin-layer chromatographic methods described in the Experimental section have been used in the separation and identification of the volatile monocarbonyl compounds from salted cod (Yurkowski and Bor deleau, 1965).

Two-dimensional separation of a mixture of monocarbonyl 2,4-dinitrophenylhydrazones are shown in Fig. 5 and 6. A mixture in the first dimension is separated into classes on magnesia-cellulose and then into individual class members on the same plate impregnated with either propylene glycol or petrolatum. When propylene glycol is used as the impregnant the *n*-alkanals and *n*-alkan-2-ones up to C_6, and *n*-alk-2-enals perhaps as high as C_8, are separated (Fig. 5), but when petrolatum is used as the impregnant the higher homologues are resolved (Fig. 6). Resolution of classes into the homologues by these systems appears better than the separation of the corresponding compounds by the two-dimensional system of Urbach (1963), where certain alkanals, alk-2-enals, and alka-2,4-dienals were separated on aluminum oxide G containing 20% silver nitrate and using 16% diethyl ether in light petroleum (b.p. 30–40°) in the first direction and in the second direction on the same plate impregnated with phenoxyethanol developed with light petroleum (b.p. 100–120°).

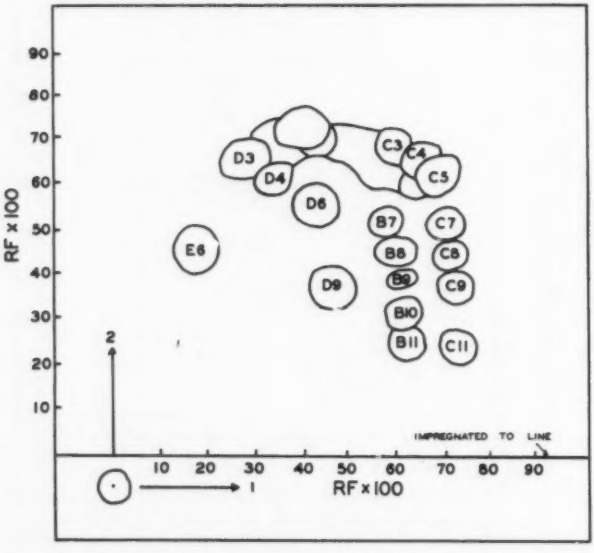

FIG. 6. Two-dimensional thin-layer chromatogram of monocarbonyl 2,4-dinitrophenylhydrazones on (1) magnesia-cellulose and (2) on same plate impregnated with petrolatum. B = *n*-alkanals; C = *n*-alkan-2-ones; D = *n*-alk-2-enals; E = *n*-hexa-2,4-dienal. The numbers represent the chain length. Amount applied, 6 μliters.

Usefulness of these two-dimensional methods is perhaps in the preliminary survey of unknown mixtures for the major components.

ACKNOWLEDGMENTS

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Carbonyl Compounds in Salted Cod. II. Separation and Identification of Volatile Monocarbonyl Compounds from Heavily Salted Cod[1]

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ABSTRACT

Volatile monocarbonyl compounds were isolated from heavily salted cod (*Gadus morhua*) as the 2,4-dinitrophenylhydrazones. These compounds were separated by thin-layer chromatography first into classes and then according to chain length. *n*-Alkanals C_{1-5} and *n*-alk-2-enals C_{5-6} were conclusively identified. *n*-Alkanals $C_{8,10}$ and *n*-alk-2-enals $C_{4,7,8}$ were tentatively identified. An unknown compound corresponding to *n*-hexanal, except in the melting point, was isolated. Formaldehyde, and to a lesser extent, acetaldehyde and propanal, were the major monocarbonyls present in salted cod.

INTRODUCTION

In the processing procedures and storage of salted cod, the quality and the flavour of the finished product is influenced by many factors such as the initial quality of the cod, the temperature, salt concentration and purity, drying conditions, sunlight, moisture, and so on. The product under unfavourable conditions can toughen in texture, darken in colour (sunburn), and develop rancid and burnt flavours and odours. This is to be expected since cod muscle lipids consist predominately of highly unsaturated phospholipids (Lovern, 1956; Notevarp, 1961), which are very susceptible to polymerisation and oxidative deterioration resulting in the formation of carbonyl compounds (Lea, 1953; 1957). Free fatty acids are probably involved in these reactions, since they are produced during preparation of salted cod by hydrolysis of phospholipids (Cardin *et al.*, 1958).

Carbonyl compounds have been studied in various fish products. Volatile carbonyls, representing one or more classes such as *n*-alkanal, 2-methylalkanal, *n*-alk-2-enal, *n*-alka-2,4-dienal, *n*-alkan-2-one, and others, were found in canned salmon (Tanikawa, 1958), pressure cooked sardine, mackerel, and flat fish

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(Ota, 1958), fresh and frozen haddock (Mangan, 1959), chill-stored cod (Groninger, 1961), heat processed herring (Hughes, 1961), and autoxidised fish oils (Chipault, 1959; Toyomizu, 1960a, b; Yu *et al.*, 1961; Wyatt and Day, 1963). Nonvolatile (or bound) and volatile (or free) monocarbonyls as classified by Gaddis *et al.* (1960) were studied in autoxidised salmon oil (Yu *et al.*, 1961; Wyatt and Day, 1963). They found that the bound monocarbonyls are apparently precursors of free monocarbonyls, which are largely responsible for the rancid odour. It was suggested that the carbonyls are involved in the rusting of frozen salmon and browning of fish meals (Wyatt and Day, 1963).

The purpose of this work was to isolate, identify, and estimate some of the volatile monocarbonyls in dry salted cod (*Gadus morhua*).

EXPERIMENTAL

Materials

Benzene, cyclohexane, heptane, and hexane were passed through a sulfuric acid–Celite column (Hornstein and Crowe, 1962), then refluxed with sodium hydroxide and distilled. Absolute methanol, ethanol, and chloroform were refluxed with trichloroacetic acid and 2,4-dinitrophenylhydrazine and distilled (Yu *et al.*, 1961). Equilibrated hexane was prepared by shaking purified hexane with excess redistilled nitromethane. Authentic monocarbonyl 2,4-dinitrophenylhydrazones were prepared as previously described (Yurkowski, 1965).

Preparation of Sample

Fourteen cod, commercially salted in 1962, were filleted by removing the skin and backbone. Fillets averaging 56 cm long were sectioned into quarters, and combined alternatively to make four representative samples. The samples were stored at 0°C until used. A sample was ground in a Wiley mill, mixed, and 1160 g of fish (equivalent to 293 g moisture and salt-free fillet) was homogenized in 2 liters of water and 5 ml of Dow Corning antifoam A in a Waring blendor.

Isolation of Volatile Monocarbonyls

The homogenate was steam distilled from a 5-liter flask through which steam was passed and which was also heated with boiling water. The vapor, containing the carbonyls, was first condensed into a 2-liter trap containing 500 ml of 4*N* sulfuric acid saturated with 2,4-dinitrophenylhydrazine. The residual carbonyl vapors were trapped in three succeeding traps containing 50 ml each of 2*N* sulfuric acid saturated with 2,4-dinitrophenylhydrazine. The traps were cooled with ice water. After collection of 1200 ml of distillate (1 hr), the traps were closed and the contents were allowed to react overnight at 5°C. The contents of the traps were combined and the 2,4-dinitrophenylhydrazones were extracted eight times with 250-ml portions of chloroform. The chloroform extract was washed with 250-ml portions of water to remove the acid. The chloroform extracts were then evaporated to dryness. The residue

was extracted twenty times with 20-ml portions of equilibrated nitromethane and reduced to dryness. The remaining dicarbonyl residue was dark red in colour. The monocarbonyl extract residue was dissolved in a known volume of ethanol.

Separation of Monocarbonyl 2,4-Dinitrophenylhydrazones

Thin-layer chromatographic methods for the separation of monocarbonyl compounds were previously described (Yurkowski, 1965).

Monocarbonyls from heavy-salted cod were separated on many activated silica gel G plates into three fractions corresponding in mobility to standard solutions of formaldehyde (fraction 1) and butanal (fraction 2) and those of higher mobility than butanal (fraction 3). The fractions were scraped off, pooled, and extracted with ethanol.

Fraction 1 was separated on propylene glycol-impregnated silica gel G plates into three main fractions (spots) which were scraped off, pooled, and eluted with heptane. They were further purified by rechromatography or by chromatography on magnesia-cellulose plates or both.

Fraction 2 was separated into classes on magnesia-cellulose plates. Individual class fractions were pooled, eluted with methanol, and rechromatographed if impure. Class fractions were then separated into individual components on propylene glycol-impregnated silica gel G plates. Pooled fractions were eluted with heptane and rechromatographed, except those corresponding to six carbons or more, which were combined later with their respective classes from fraction 3.

Fraction 3 was separated into classes and individual compounds in the same manner as fraction 2, except that the petrolatum-impregnated plates replaced those impregnated with propylene glycol. The pooled fractions from petrolatum-impregnated plates were eluted with methanol.

Removal of Impregnant from Fractions

Propylene glycol in isolated fractions was dissolved in a minimum amount of ethanol, diluted with excess distilled water and the hydrazones were extracted with heptane or hexane.

Petrolatum in isolated fractions was removed in a 2.5-cm column containing 25 g alumina mixed with 1.5 ml of water (Mookerjee and Chang, 1963). The fractions dissolved in hexane were applied on the columns, the petrolatum was eluted with 125 ml of hexane and the hydrazones with benzene–hexane (1:1), collecting only the yellow hydrazone band.

Identification of the Monocarbonyls

To determine the identity of classes and individual homologues authentic compounds were chromatographed on the same plate with the unknowns. For comparison of their mobilities, activated silica gel G plates were also used to

further check the separation and identity of individual homologues. The class of a compound was further determined from its absorption spectra in chloroform using a Beckman DK 2 recording spectrophotometer. Where possible, uncorrected melting points were determined on the unknown, authentic, and mixed compounds.

Semiquantitative Estimation of Monocarbonyls

After purification by rechromatography the isolated compounds were dissolved in known volumes of chloroform and the optical densities were determined at their absorption maxima. The concentration of the monocarbonyls was estimated using molar absorptivity indices of 22,500 and 27,500 for alkanals and alk-2-enal, respectively (Wyatt and Day, 1963).

RESULTS AND DISCUSSION

The data on the isolation and identification of volatile monocarbonyl compounds as 2,4-dinitrophenylhydrazones from heavily salted cod are presented in Table I. Thirteen compounds, not present in the blank, were isolated, of which seven were conclusively and five tentatively identified; one was not identified. *n*-Octanal, *n*-decanal, propenal, *n*-hept-2-enal, and *n*-oct-2-enal were identified on the basis of their maximum absorption spectra and chromatographic behaviour. The unknown compound corresponded to *n*-hexanal in all the physical characteristics determined, except the melting point which was too low.

The blank contained other monocarbonyl compounds, which were identified as *n*-alkan-2-ones C_{3-8}, *n*-alkanals $C_{7,9,11}$ and *n*-alk-2-enal C_9 by their absorption spectra and chromatographic mobility. These compounds, except the *n*-alkan-2-ones, may occur in salted cod in very low concentrations since they do occur in autoxidised salmon oils (Wyatt and Day, 1963; Yu *et al.*, 1961).

The nature of volatile monocarbonyl compounds present in salted cod resembles somewhat that in autoxidised calamary (Toyomizu, 1960a, b) and salmon (Wyatt and Day, 1963) oils.

In salted cod, as in whole dried milk (Corbin *et al.*, 1960), formaldehyde is the major monocarbonyl present. Acetaldehyde and propanal also form a substantial part of the monocarbonyls.

No attempt has been made to determine the contribution of the monocarbonyl compounds to the flavour of salted cod. However, it has been observed that the unabsorbed odour during distillation resembled hydrogen sulfide and after distillation the fish residue still had a faint fishy odour. It is interesting to note that the residue from lightly salted (Gaspé-cure) cod, after removal of the carbonyls, had a mushroom-like odour, presumably caused by cyclic acetals, i.e. 1,3-dioxalans (Hoffmann, 1961). Forss *et al.* (1960) detected this odour in butter.

Mechanisms for the formation of some monocarbonyls by autoxidation of unsaturated lipids have been discussed (Keeney, 1961) on the basis of the

TABLE I. Identification data for the 2,4-dinitrophenylhydrazones of volatile monocarbonyl compounds from heavily salted cod.

2,4-DNP-hydrazones	Methods of isolation[a]	μM carbonyl/kg[b]	Maximum absorptions (Mμ)		Melting Points (°C)		
			Known	Found	Known	Found	Mixture
n-methanal	SPM	$C_1 \gg C_2$[c]	345	345	167–69	167–68	167–68
n-ethanal	SPM	$C_2 = C_3$[c]	355	354	167–69	167	
n-propanal	SMP	8.02	357	355–57	155–56	153–55	152–54
n-butanal	SMP	1.99	357	356	122	115–16	118–21
n-pentanal	SMV	2.47	357	356–58	107–08	104–06	
Unknown	SMV	3.12	358[d]	357–58	108–09[d]	99–102[e]	
n-octanal	SMV	0.54	358	358			
n-decanal	SMV	0.23	357	358			
n-but-2-enal	SMP	1.02	372–73	371			
n-pent-2-enal	SMV	0.42	373[d]	371–74	150–54[d]	148–51	
n-hexa-2-enal	SMV	0.48	374	372–73	143–44	137–40	
n-hept-2-enal	SMV	0.26		372			
n-oct-2-enal	SMV	0.13	373[d]	372			

[a]M = magnesia-cellulose, S = activated silica gel G, P = propylene glycol-impregnated, and V = petrolatum-impregnated silica gel G plates.
[b]Calculated on dry salt-free basis less blank.
[c]Visual comparison of spot density.
[d]Values obtained from Yu *et al.* (1961).
[e]Compares in all physical characteristics determined to *n*-hexanal, except the melting point was low.

hydroperoxide theory (Farmer *et al.*, 1942). The formation of some monocarbonyl compounds in salted cod might be explained on that basis.

The similar relative concentrations of volatile acids, viz. formic, acetic, propionic, butyric, and valeric acids, in salted cod (Bordeleau and Vaillancourt, MS, 1964) suggest that the monocarbonyl compounds are their precursors.

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Some Methods for Estimating Ocean Mortality of Pacific Salmon and Applications[1,2]

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ABSTRACT

Three methods for estimating oceanic natural mortality rates of Pacific salmon under certain survival conditions are presented. Estimates of ocean mortality rates of Bristol Bay and Karluk sockeye are given. The mortality rate during the year immediately preceding the last 2 months of ocean life of Bristol Bay sockeye that migrated to sea as 3-freshwater fish in 1956 and returned as mature 3-ocean fish in 1959 is estimated to be 28.9%. The average ocean mortality rate during the penultimate year of life of 3-ocean Bristol Bay sockeye is estimated to be 19.5% for the years 1956–57 to 1960–61. The natural mortality rate during the third year of ocean life of 3-freshwater Karluk sockeye is estimated to be 28.2%. Corresponding estimates of average monthly instantaneous mortality rates are 0.028, 0.018, and 0.031, respectively.

INTRODUCTION

An impressive number of papers on estimates of oceanic natural mortality rates of Pacific salmon has become available within recent years. Parker (1962, 1963) lists these papers in his substantial contributions to the knowledge of this important biological statistic. The purpose of this paper is to add to the knowledge of ocean mortality rates of salmon by presenting a few methods for estimating such rates and applying these methods to existing data.

MATURITY SCHEDULE METHOD

Derivation of Equations

Several important sockeye salmon runs of North America are composed almost entirely of fish that migrate to sea in their second or third year of life as 2- or 3-freshwater fish and spend 2 or 3 years in the ocean before returning to their natal streams as mature 2- or 3-ocean fish. I shall use a model that simulates survival conditions during a portion of the ocean life history of such salmon runs to illustrate the derivation of equations for estimating an oceanic natural mortality rate from data on maturity schedules and returns.

The seaward migration of 2- and 3-freshwater fish takes place in late May or early June. Approximately 2 years later, the survivors, known as 2-ocean fish, fall into one of four maturity-age categories: mature 4.2 fish, immature

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4.2 fish, mature 5.3 fish, and immature 5.3 fish. The first numeral represents the year of life of the fish, and the second numeral represents the year of life in which the fish migrated to sea.

The numbers of 2-ocean fish of the two freshwater age groups on May 1 of year x at sea are represented by $N_{4.2}$ and $N_{5.3}$. The proportions of mature 2-ocean fish in the two freshwater age groups are denoted by $p_{4.2}$ and $p_{5.3}$. Thus, the numbers of mature and immature 2-ocean fish of the two freshwater age groups on May 1 of year x at sea are represented as follows:

Freshwater age group	Number of 2-ocean fish	
	Mature	*Immature*
2	$N_{4.2}\ p_{4.2}$	$N_{4.2}\ (1-p_{4.2})$
3	$N_{5.3}\ p_{5.3}$	$N_{5.3}\ (1-p_{5.3})$

The mature 2-ocean fish return to their natal stream on July 1 of year x. The survival rates for the two freshwater age groups of mature 2-ocean fish between May 1 and July 1 of year x are represented by s_{m2} and s_{m3}. The returns of 2-freshwater, 2-ocean fish ($R_{4.2}$) and 3-freshwater, 2-ocean fish ($R_{5.3}$) on July 1 of year x are as follows:

$$R_{4.2} = N_{4.2}\ p_{4.2}\ s_{m2} \tag{1}$$

and

$$R_{5.3} = N_{5.3}\ p_{5.3}\ s_{m3} \tag{2}$$

The immature 2-ocean fish spend an additional year at sea before returning to their natal stream as 3-ocean fish. The survival rates for the two freshwater age groups of immature 2-ocean fish between May 1 of year x and May 1 of year $x + 1$ (at which time they are mature 3-ocean fish) are represented by s_{i2} and s_{i3}. The numbers of surviving 2-freshwater, 3-ocean fish ($N_{5.2}$) and 3-freshwater, 3-ocean fish ($N_{6.3}$) on May 1 of year $x + 1$ are as follows:

$$N_{5.2} = N_{4.2}\ (1-p_{4.2})\ s_{i2}$$

and

$$N_{6.3} = N_{5.3}\ (1-p_{5.3})\ s_{i3}$$

The 3-ocean fish return to their natal stream on July 1 of year $x + 1$. The survival rate for 2-freshwater, 3-ocean fish between May 1 and July 1 of year $x + 1$ is assumed to be s_{m2}, the same as the survival rate for mature 2-freshwater, 2-ocean fish during the corresponding 2-month period in year x. The survival rate for 3-freshwater, 3-ocean fish between May 1 and July 1 of year $x + 1$ is assumed to be s_{m3}, the same as the survival rate for mature 3-freshwater, 2-ocean fish during the corresponding 2-month period in year x. These survival conditions are analogous to those simulated in Population

Models 1, 3, and 4 of my Karluk sockeye ocean mortality analysis (Fredin, MS, 1963).

The returns of 2-freshwater, 3-ocean fish ($R_{5.2}$) and 3-freshwater, 3-ocean fish ($R_{6.3}$) on July 1 of year $x + 1$ are as follows:

$$R_{5.2} = N_{4.2}\ (1-p_{4.2})\ s_{t2}\ s_{m2} \tag{3}$$

and

$$R_{6.3} = N_{5.3}\ (1-p_{5.3})\ s_{t3}\ s_{m3} \tag{4}$$

Dividing equation (3) by (1) and equation (4) by (2), we obtain

$$\frac{R_{5.2}}{R_{4.2}} = \frac{(1-p_{4.2})\ s_{t2}}{p_{4.2}} \tag{5}$$

and

$$\frac{R_{6.3}}{R_{5.3}} = \frac{(1-p_{5.3})\ s_{t3}}{p_{5.3}} \tag{6}$$

Equation (5) can be used to estimate s_{t2} when data on returns of 4.2 and 5.3 fish and an estimate of $p_{4.2}$ are available. Similarly, equation (6) can be used to estimate s_{t3} when data on returns of 5.3 and 6.3 fish and an estimate of $p_{5.3}$ are available.

Application of Method

Table I shows the numbers of 4.2, 5.3, 5.2, and 6.3 fish in the combined sockeye runs to the Naknek-Kvichak, Nushagak, Egegik, and Ugashik river systems in Bristol Bay, Alaska, between 1956 and 1961, and the numbers of 2- and 3-ocean sockeye in the combined run in 1962. For 5 of the first 6 years, other age groups of sockeye accounted for less than 1% of the total Bristol Bay sockeye runs. In 1959, other age groups accounted for approximately 3% of the total run. The age composition data that are available for 1962 indicate that less than 1% of the total run in that year was composed of fish of age groups other than 4.2, 5.3, 5.2, and 6.3. Thus, for all practical purposes, the Bristol Bay sockeye runs can be considered to be composed of fish of the same four age groups as in the model run used to derive equations (5) and (6).

The numbers of fish shown in Table I do not necessarily represent the total returns of Bristol Bay sockeye, since they do not include the catches of Bristol Bay sockeye by the Japanese gill net fishery in the North Pacific Ocean. Tagging and racial studies by Canadian, Japanese, and United States fisheries scientists (Hartt, 1962; Fukuhara *et al.*, 1962; Margolis, 1963; Mosher, 1963; INPFC, 1961, 1962, 1963a) have shown that Bristol Bay sockeye occur in considerable quantities in high seas areas where Japanese gill net fishing takes place. The precise number of Bristol Bay sockeye, mature or immature, taken by the high seas gill net fishery each year is not known. Available data indicate only that in some years several million Bristol Bay sockeye are caught on the high seas.

TABLE I. Numbers of 4.2, 5.3, 5.2, and 6.3 fish in the Bristol Bay sockeye runs, 1956–61, and numbers of 2- and 3-ocean fish in the 1962 run. (All figures in millions of fish. Data for 1956–61 from Documents 491 and 602, International North Pacific Fisheries Commission; data for 1962 from Alaska Department of Fish and Game, Fisheries Research Institute, University of Washington, and Bureau of Commercial Fisheries, U.S. Fish and Wildlife Service.)

Year	Size of run						
	Age group		Subtotal	Age group		Subtotal	Total
	4.2	5.3	2-ocean fish	5.2	6.3	3-ocean fish	
1956	12.105	8.472	20.577	2.385	1.604	3.989	24.566
1957	0.430	2.261	2.691	6.367	2.086	8.453	11.144
1958	1.346	1.485	2.831	1.310	1.331	2.644	5.475
1959	4.506	5.513	10.019	1.071	0.787	1.858	11.877
1960	30.285	1.732	32.017	2.590	1.322	3.912	35.929
1961	1.405	6.445	7.850	9.894	0.485	10.379	18.229
1962	[a]	[a]	7.000	[a]	[a]	3.147	

[a]Incomplete data

In such years, of course, the numbers of fish in the runs to Bristol Bay are considerably less than they would be if none of the sockeye had been caught on the high seas.

Statistical yearbooks of the International North Pacific Fisheries Commission (1956–62) list the Japanese high seas gill net catches of sockeye by 5-degree intervals of longitude and month of fishing. For the years 1956–60, Manzer *et al.* (MS, 1963) present age composition data, by monthly periods, for sockeye taken by the high seas gill net fishery east of 170° E long. Their age composition data are shown here in Table II.

Age composition data shown in Table II and catch statistics from the INPFC statistical yearbooks are used to estimate the Japanese high seas gill net catches of 4.2, 5.3, 5.2, and 6.3 sockeye in various areas of the North Pacific Ocean during various months for the years 1956–62. Average percentage age composition data for 1956–60 are used for compiling the high seas catches by age groups for 1961 and 1962. The compilation is shown in Table III.

Judging from data on migration rates of Bristol Bay sockeye (INPFC, 1961, app. 1, fig. 16) and the results of tagging and racial studies referred to previously, the areas and months for which catch statistics are shown in Table III probably represent the principal locations and times that mature Bristol Bay sockeye are caught by the Japanese high seas gill net fishery.

The catch statistics given in Table III indicate that, in 1958, small numbers of 2-ocean sockeye were caught by the high seas gill net fishery in the areas and months listed. The data also indicate that, in 1959, the high seas catches of

TABLE II. Estimated percentage age composition of sockeye caught by the Japanese high seas gill net fishery east of 170° E long, by month. (Data from Manzer *et al.*, MS, 1963.)

Year	Month	Age group		Sub-total 2-ocean	Age group		Sub-total 3-ocean	Total	Other age groups
		4.2	5.3		5.2	6.3			
1956	May	13	28	41	21	30	51	92	8
	June	24	34	58	15	21	36	94	6
	July	24	41	65	14	14	28	93	7
1957	May	1	15	16	38	41	79	95	6
	June	2	22	24	40	32	72	96	4
	July	8	27	35	30	29	59	94	6
1958	May	2	13	15	20	51	71	86	14
	June	6	35	41	17	28	45	86	14
	July	–	–	–	–	–	–	–	–
	Aug.	25	53	78	7	6	13	91	9
1959	May	6	22	28	19	34	53	81	19
	June	13	42	55	11	18	29	84	16
	July	23	50	73	7	7	14	87	13
1960	May	10	16	26	31	31	62	88	12
	June	43	11	54	16	25	41	95	5
	July	27	50	77	5	11	16	93	7
	Aug.	27	66	93	2	3	5	98	2
Averages:									
1956–60	May	6.4	18.8	25.2	25.8	37.4	63.2	88.4	
	June	17.6	28.8	46.4	19.8	24.8	44.6	91.0	
	July	20.5	42.0	62.5	14.0	15.2	29.2	91.7	
	Aug.	26.0	59.5	85.5	4.5	4.5	9.0	94.5	

3-ocean sockeye were relatively small. If it is assumed that negligible numbers of 2-ocean Bristol Bay sockeye were caught on the high seas in 1958, and also that negligible numbers of 3-ocean Bristol Bay sockeye were caught offshore in 1959, the runs of these two ocean age groups to Bristol Bay in 1958 and 1959, respectively, can be considered to represent the total returns. Under these conditions, data on the sizes of the Bristol Bay runs of 2-ocean fish in 1958 and 3-ocean fish in 1959 can be used for estimating ocean survival and mortality rates from equation (5) or (6).

To use equation (5) or (6) to estimate ocean survival and mortality rates for Bristol Bay sockeye, it is necessary to assign values to the proportions of 2-ocean (4.2 or 5.3) Bristol Bay fish that matured in 1958. To my knowledge, there are no estimates available for the proportions of Bristol Bay sockeye that matured as 2-ocean fish in 1958 or any other year. In this circumstance, I will use certain maturity schedule data for Karluk sockeye (Fredin, MS, 1963) to represent the maturity schedules of Bristol Bay sockeye.

As indicated previously, the survival conditions which I assumed in deriving equations (5) and (6) are analogous to those simulated by Population Models 1, 3, and 4 in my Karluk sockeye study. In that study, Population Model 1

TABLE III. Estimated catches of 4.2, 5.3, 5.2, and 6.3 sockeye by the Japanese gill net fishery in various areas and months of fishing in the North Pacific Ocean. (Data from INPFC statistical yearbooks and Manzer *et al.*, MS, 1963.)

Area	Months	Year	Catch in millions of fish						
			Age group		Sub-total 2-ocean fish	Age group		Sub-total 3-ocean fish	Total
			4.2	5.3		5.2	6.3		
175° W to 180°	June–July	1956	0.183	0.279	0.462	0.112	0.142	0.254	0.716
		1957	0.056	0.588	0.644	1.060	0.851	1.911	2.555
		1958	0	0	0	0	0	0	0
		1959	0	0	0	0	0	0	0
		1960	1.134	0.326	1.460	0.418	0.655	1.073	2.533
		1961	0.765	1.252	2.017	0.861	1.079	1.940	3.957
		1962	0.031	0.051	0.082	0.035	0.044	0.079	0.161
180° to 175° E	May–June	1956	0.365	0.567	0.932	0.295	0.416	0.711	1.643
		1957	0.047	0.518	0.565	0.952	0.772	1.724	2.289
		1958	0	0.002	0.002	0.003	0.009	0.012	0.014
		1959	0.038	0.123	0.161	0.032	0.053	0.085	0.246
		1960	0.405	0.104	0.509	0.151	0.236	0.387	0.896
		1961	0.132	0.216	0.348	0.148	0.185	0.333	0.681
		1962	0.070	0.115	0.185	0.080	0.101	0.181	0.366
175° E to 170° E	May	1956	0.112	0.241	0.353	0.181	0.258	0.439	0.792
		1957	0.020	0.292	0.312	0.741	0.800	1.541	1.853
		1958	0.011	0.074	0.085	0.113	0.289	0.402	0.487
		1959	0.023	0.085	0.108	0.073	0.131	0.204	0.312
		1960	0.021	0.033	0.054	0.064	0.064	0.128	0.182
		1961	0.035	0.103	0.138	0.141	0.204	0.345	0.483
		1962	0.028	0.082	0.110	0.113	0.164	0.277	0.387
Totals		1956	0.660	1.087	1.747	0.588	0.816	1.404	3.151
		1957	0.123	1.398	1.521	2.753	2.423	5.176	6.697
		1958	0.011	0.076	0.087	0.116	0.298	0.414	0.501
		1959	0.061	0.208	0.269	0.105	0.184	0.289	0.558
		1960	1.560	0.463	2.023	0.633	0.955	1.588	3.611
		1961	0.932	1.571	2.503	1.150	1.468	2.618	5.121
		1962	0.129	0.248	0.377	0.228	0.309	0.537	0.914

simulates a constant monthly instantaneous mortality rate throughout ocean life. In Population Model 3, it is assumed that the instantaneous mortality rate is greatest during the first month of ocean residence, decreases geometrically (by a factor of 0.5) during each of the next 5 months of ocean life, and remains constant during the sixth and each succeeding month of ocean life. Population Model 4 simulates a catastrophic, age-specific first-month instantaneous mortality rate and a constant age-independent mortality rate in the second and succeeding months of ocean residence.

Using Population Models 1, 3, 4 and the releases and returns of seven groups of 3-freshwater Karluk sockeye marked as smolts in the late 1920's and early 1930's (Barnaby, 1944), the estimated average percentages of 3-freshwater Karluk sockeye that mature as 1- and 2-ocean fish are as follows (Fredin, MS, 1963, tables 6, 14, and 18):

Population model	Estimated average per cent maturing	
	1-ocean fish	*2-ocean fish*
1	1.35	51.41
3	2.35	59.78
4	2.11	57.30

Adjusting for the small proportions of sockeye that mature as 1-ocean fish (equation 15 of Fredin, MS, 1963), the estimated average percentages of 3-freshwater, 2-ocean Karluk sockeye at sea that mature as 2-ocean fish are as follows: Population Model 1—52.1%; Population Model 3—61.2%; and Population Model 4—58.5%. The mean of these three averages is 57.3%.

Using 57.3% as the proportion of the 3-freshwater, 2-ocean Bristol Bay sockeye at sea on May 1 of 1958 that returned to Bristol Bay as 2-ocean fish in 1958, we estimate s_{t3} from equation (6) as follows:

$$R_{5.3\ (1958)} = 1.485 \text{ million fish; } R_{6.3\ (1959)} = 0.787 \text{ million fish;}$$

$$s_{t3} = \frac{0.787\ (0.573)}{1.485\ (1 - 0.573)} = 0.711$$

The estimated survival rate of 0.711 is equivalent to an instantaneous ocean mortality rate of approximately 0.34 for the 12-month period from May 1, 1958, to May 1, 1959. The estimated average monthly instantaneous ocean mortality rate during this period is approximately 0.028.

OCEAN IMMATURES METHOD

Derivation of Equations

To illustrate the derivation of equations for estimating an oceanic natural mortality rate of a salmon run from indices of abundance of immature fish at sea and returns of mature fish, I shall use as a model a sockeye run whose members spend either 2 or 3 years in the ocean before returning to their natal stream as mature fish.

A seaward migration of smolts is considered to take place on June 1 of year 1. Fourteen months later — that is, on August 1 of year 2 — the survivors are immature 1-ocean fish. Their abundance at that time is $N_{1.2}$ (the first numeral in the subscript represents the ocean age of the fish, and the second numeral represents the calendar year). Approximately 8 months later, or 22 months after their seaward migration, the survivors are 2-ocean fish. Many of the 2-ocean fish mature and return to their natal stream on July 1 of year 3, 25 months after the beginning of ocean life.

Denoting the proportion of the $N_{1.2}$ fish that mature as 2-ocean sockeye by $p_{1.2}$, and their survival rate between August 1 of year 2 and July 1 of year 3 by s, the return, $R_{2.3}$, of mature 2-ocean sockeye on July 1 of year 3 is as follows:

$$R_{2.3} = N_{1.2}\ p_{1.2}\ s. \qquad (7)$$

The immature 2-ocean fish remain at sea where, on August 1 of year 3 (or 26 months following their seaward migration), their abundance is $N_{2.3}$. The quantity $N_{2.3}$ is equivalent to $N_{1.2}\ (1 - p_{1.2})\ s_o$, where $(1 - p_{1.2})$ is the proportion of the $N_{1.2}$ sockeye that do not mature as 2-ocean fish, and s_o is the survival rate between August 1 of year 2 and August 1 of year 3 for the $N_{1.2}\ (1 - p_{1.2})$ fish. The quantity, s_o, is referred to as the survival rate during the penultimate year of life of 3-ocean sockeye.

In the spring of year 4, the survivors are 3-ocean fish. All of the 3-ocean fish mature and return to their natal stream on July 1 of year 4, 37 months after the beginning of ocean residence. Denoting the survival rate between August 1 of year 3 and July 1 of year 4 by s, the return, $R_{3.4}$, of 3-ocean fish on July 1 of year 4 is as follows:

$$\begin{aligned} R_{3.4} &= N_{2.3}\ s \\ &= N_{1.2}\ (1 - p_{1.2})\ s_o\ s. \end{aligned} \tag{8}$$

In addition to the immature 2-ocean fish at sea on August 1 of year 3, there is also present a number of immature 1-ocean fish. The latter are survivors from the smolt migration that takes place on June 1 of year 2. Their abundance on August 1 of year 3 is $N_{1.3}$.

The return, $R_{2.4}$, of mature 2-ocean fish on July 1 of year 4 is as follows:

$$R_{2.4} = N_{1.3}\ p_{1.3}\ s \tag{9}$$

where $p_{1.3}$ is the proportion of the $N_{1.3}$ fish that mature as 2-ocean sockeye, and s is the survival rate between August 1 of year 3 and July 1 of year 4.

The abundance of immature 2-ocean fish on August 1 of year 4 is $N_{2.4}$. These are survivors from the smolt migration of June 1 of year 2. Their abundance is equivalent to $N_{1.3}\ (1 - p_{1.3})\ s_o$, where s_o is the survival rate between August 1 of year 3 and August 1 of year 4 for the $N_{1.3}\ (1 - p_{1.3})$ fish.

The return, $R_{3.5}$, of 3-ocean fish on July 1 of year 5 is as follows:

$$\begin{aligned} R_{3.5} &= N_{2.4}\ s \\ &= N_{1.3}\ (1 - p_{1.3})\ s_o\ s \end{aligned} \tag{10}$$

where s is the survival rate between August 1 of year 4 and July 1 of year 5.

Dividing equation (9) by (8) and transposing terms, we obtain

$$p_{1.3} = \frac{R_{2.4}}{R_{3.4}}\ \frac{N_{2.3}}{N_{1.3}}. \tag{11}$$

Dividing equation (10) by (9) and transposing terms, we obtain

$$s_o = \frac{R_{3.5}\ p_{1.3}}{R_{2.4}\ (1 - p_{1.3})}. \tag{12}$$

If we have indices of abundance of immature 1- and 2-ocean fish at sea on August 1 of year 3, such that $I_{1.3} = kN_{1.3}$ and $I_{2.3} = kN_{2.3}$, equation (11) can be expressed as follows:

$$p_{1.3} = \frac{R_{2.4}}{R_{3.4}} \frac{I_{2.3}}{I_{1.3}}. \tag{13}$$

The equivalent of $p_{1.3}$ can be used in equation (12) to estimate s_0.

In this model, the survival rate during the last 11 months of ocean life of mature fish is assumed to be constant from year to year and the same for 2- and 3-ocean sockeye.

Application of Method

If it is assumed that all 4.2 and 5.3 sockeye (2-ocean fish) and all 5.2 and 6.3 sockeye (3-ocean fish) taken by the Japanese high seas gill net fishery between 170° E and 175° E in May, between 175° E and 180° in May and June, and between 180° and 175° W in June and July are mature Bristol Bay fish, we can add the catch statistics of Tables I and III to obtain estimates of the total returns of 2- and 3-ocean Bristol Bay sockeye (R_2 and R_3) between 1956 and 1962. These estimates are given in Table IV.

Tagging studies conducted by the Fisheries Research Institute, University of Washington, since 1956 in the North Pacific Ocean south of Adak Island have shown that immature 1- and 2-ocean Bristol Bay sockeye occur there during the months of July and August (Hartt, 1962; INPFC, 1956–61). Fishing by purse seine vessels to capture salmon for tagging south of Adak Island has also provided annual indices of abundance (average catch per seine set) of immature 1- and 2-ocean sockeye occurring there. These indices of abundance,

Table IV. Estimated total returns of 2- and 3-ocean Bristol Bay sockeye, 1956–62. (Data from Tables I and III.)

Year	Return (millions of fish)		
	2-ocean (4.2 and 5.3) R_2	3-ocean (5.2 and 6.3) R_3	Total
1956	22.324	5.393	27.717
1957	4.212	13.629	17.841
1958	2.918	3.058	5.976
1959	10.288	2.147	12.435
1960	34.040	5.500	39.540
1961	10.353	12.997	23.350
1962	7.377	3.684	11.061

TABLE V. Average catch per seine set of immature sockeye south of Adak Island, 1956–62.

Year	Average catch per set (no. of fish)	
	Immature 1-ocean sockeye I_1	Immature 2-ocean sockeye I_2
1956	16.4	29.2
1957	4.3	1.7
1958	38.7	3.3
1959	126.1	14.5
1960	12.9	28.6
1961	19.7	4.1
1962	20.4	10.4

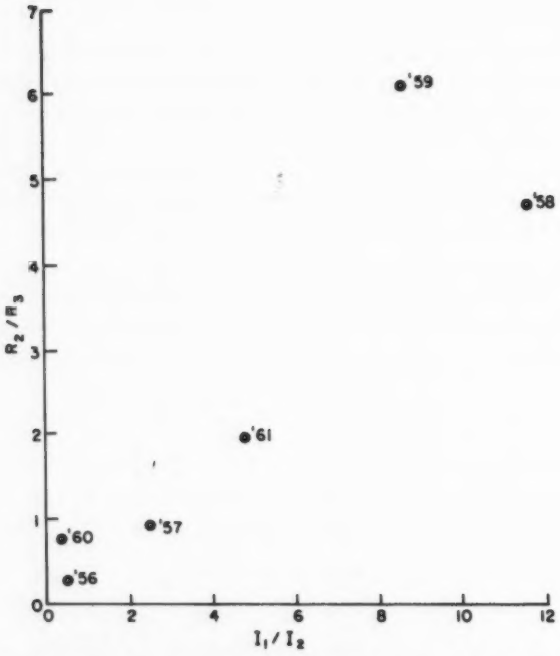

FIG. 1. Relation between ratio of estimated returns of 2- and 3-ocean Bristol Bay sockeye (R_2/R_3) and ratio of relative abundance of immature 1- and 2-ocean sockeye at sea in preceding year (I_1/I_2). (Year at sea indicated.)

TABLE VI. Ratios of relative abundance of immature 1- and 2-ocean sockeye south of Adak Island, 1956–61, and ratios of estimated returns of 2- and 3-ocean Bristol Bay sockeye, 1957–62.

Year at sea	Ratio of relative abundance I_1/I_2	Year of return	Ratio of estimated returns R_2/R_3
1956	0.56	1957	0.31
1957	2.53	1958	0.95
1958	11.73	1959	4.79
1959	8.70	1960	6.19
1960	0.45	1961	0.80
1961	4.80	1962	2.00

I_1 and I_2, respectively, are given in Alaska Department of Fish and Game (1963, table 8), and are shown here in Table V.

The ratios of relative abundance of immature 1- and 2-ocean sockeye for the years 1956–61 and the ratios of estimated returns of 2- and 3-ocean Bristol Bay sockeye for the years 1957–62 are shown in Table VI, and are plotted in Fig. 1.

The relation between R_2/R_3 and I_1/I_2 is clearly positive and, statistically, is significant at about the 1% confidence level (r, the correlation coefficient, equals 0.91). The relation indicates that the indices of abundance of immature 1- and 2-ocean sockeye obtained by purse seine fishing south of Adak Island in the summer are representative of the relative numbers of immature 1- and 2-ocean Bristol Bay sockeye at sea on August 1, and can be used to estimate ocean survival of Bristol Bay sockeye from equations (13) and (12).

The variation of the relation between R_2/R_3 and I_1/I_2 is probably largely due to year-to-year changes in the availability of immature 1- and 2-ocean Bristol Bay sockeye to the purse seine vessels fishing south of Adak Island, differences in the fishing efficiency of purse seines from year to year, and fluctuations in the abundance of other sockeye stocks south of Adak Island. Because of the variation that exists, I will use average annual indices of abundance of immature 1- and 2-ocean sockeye south of Adak Island and average annual returns of 2- and 3-ocean Bristol Bay sockeye, rather than data for individual years, to estimate a survival rate for 3-ocean Bristol Bay sockeye during the 12 months immediately preceding their last 11 months of ocean life.

The average annual catches per seine set of immature 1- and 2-ocean sockeye south of Adak Island for the 5 years from 1956 to 1960 were 39.68 and 15.46 fish, respectively (Table V). The estimated average annual returns of 2- and 3-ocean Bristol Bay sockeye between 1957 and 1961 were 12,362 and

7.466 million fish, respectively (Table IV). The estimated average annual return of 3-ocean Bristol Bay sockeye between 1958 and 1962 was 5.477 million fish (Table IV).

Using the average annual indices of abundance of immature 1- and 2-ocean sockeye south of Adak Island between 1956 and 1960, and the estimated average annual returns of 2- and 3-ocean Bristol Bay sockeye between 1957 and 1961, we obtain an estimate of the average proportion of Bristol Bay sockeye that matured as 2-ocean fish in the years 1957–61 from equation (13) as follows:

$$\text{Av. } p_{1\ (1956\text{–}60)} = \left(\frac{12.362}{7.466}\right)\left(\frac{15.46}{39.68}\right) = 0.645.$$

Putting the estimate of 0.645 in equation (12), together with the estimated average annual returns of 2-ocean Bristol Bay sockeye between 1957 and 1961 and of 3-ocean Bristol Bay sockeye between 1958 and 1962, we obtain an estimate of the average annual survival rate during the penultimate year of life of 3-ocean Bristol Bay sockeye for the years 1956–57 to 1960–61. The estimate is as follows:

$$\text{Av. } s_{o\ (1956\text{–}57\ \text{to}\ 1960\text{–}61)} = \left(\frac{5.477}{12.362}\right)\left(\frac{0.645}{0.355}\right) = 0.805.$$

The estimated average annual survival rate during the penultimate year of life of 3-ocean Bristol Bay sockeye is equivalent to an instantaneous mortality rate of approximately 0.22. The corresponding average monthly instantaneous mortality rate is approximately 0.018.

SMOLT INDICES METHOD

Derivation of Equations

I shall use a salmon run whose members have an ocean life span of 2 or 3 years as a model for deriving equations for estimating oceanic natural mortality rates from indices of smolt abundance and returns of 2- and 3-ocean fish.

The number of smolts migrating to sea in any year, x, is represented by N_x. It is assumed that an index of abundance of smolts is obtained for each seaward migration such that $I_x = kN_x$.

The survival rate during the first 2 years of ocean life, that is, from the time of seaward migration to the time of return of mature 2-ocean fish, is s. The proportion of smolts that mature as 2-ocean fish is p_x. The return, R_{2x}, of 2-ocean fish is as follows:

$$R_{2x} = N_x p_x\, s. \tag{14}$$

The survival rate during the third year of ocean life for those fish that spend 3 years at sea before returning to their natal stream is s'. The return, R_{3x}, of 3-ocean fish is as follows:

$$R_{3x} = N_x\ (1 - px)\ s\ s' \tag{15}$$
$$= N_x\ s\ s' - N_x\ p_x\ s\ s'$$
$$= N_x\ s\ s' - R_{2x}\ s'.$$

Substituting I_x/k for N_x in equation (15), we obtain

$$R_{3x} = \frac{I_x}{k}\ s\ s' - R_{2x}\ s'. \tag{16}$$

Dividing both sides by I_x, we obtain,

$$\frac{R_{3x}}{I_x} = \frac{s\ s'}{k} - \frac{R_{2x}}{I_x}\ s'. \tag{17}$$

Equation (17) is linear in form. The slope of the line is s' and the intercept is ss'/k. Assuming that s and s' are constant and provided that data on relative abundance of smolts and returns of 2- and 3-ocean fish are available for a series of years, equation (17) can be used to estimate s' by linear regression methods. A measure of k, of course, would also permit an estimate to be made of s.

Application of Method

I will use a portion of the data presented by Barnaby (1944) on the releases and returns of marked Karluk sockeye to illustrate an application of equation (17) for estimating ocean mortality rates of a salmon run.

Practically all of the members of six groups (Group No. 5, 9, 13, 16, 19, and 23 as shown in table 3 of Fredin, MS, 1963) of 3-freshwater Karluk sockeye marked by Barnaby returned to Karluk River after 2 or 3 years of ocean residence. The releases and returns of these groups are as follows:

Group No.	Number of smolts marked	Estimated returns (number of fish)			
		1-ocean R_1	2-ocean R_2	3-ocean R_3	4-ocean R_4
5	14,676	28	1,730	725	0
9	14,923	42	1,587	808	17
13	41,403	39	3,845	4,857	46
16	6,275	13	833	339	11
19	9,381	0	1,256	926	45
23	25,394	7	3,208	1,441	0

Assuming that 10% of the marked fish represents the indices of abundance of smolts in the six groups, and ignoring the returns of 1- and 4-ocean fish, the

indices of abundance of smolts and the variables used in a regression analysis are as follows:

I_x	R_{2x}/I_x	R_{3x}/I_x
1468	1.18	0.49
1492	1.06	0.54
4140	0.93	1.17
628	1.33	0.54
938	1.34	0.99
2539	1.26	0.57

The regression of R_{3x}/I_x on R_{2x}/I_x is given by the equation,

$$\frac{R_{3x}}{I_x} = 1.494 - 0.657 \frac{R_{2x}}{I_x}.$$

The slope, 0.657, represents the survival rate during the third year of ocean life of marked 3-freshwater Karluk sockeye that mature as 3-ocean fish. This rate corresponds to an instantaneous mortality rate of approximately 0.42. Adjusting for the effects of marking after the manner of Ricker (1962), that is, dividing 0.42 by 1.27, the instantaneous mortality rate of unmarked fish is estimated to be 0.33. The estimated ocean survival rate of unmarked fish is approximately 71.8%. Using 10.67 months as the duration of the third year of ocean life of 3-freshwater Karluk sockeye (table 2 of Fredin, MS, 1963), the average monthly instantaneous mortality rate is estimated to be 0.031.

The intercept, 1.494, represents the product of the survival rates during the first 2 years and third year of ocean residence divided by the proportion (10%) of the smolt migrations represented by the indices of abundance. Solving for s, the survival rate during the first 2 years of ocean residence of marked 3-freshwater Karluk sockeye, we obtain

$$s = \frac{1.494\ (0.10)}{0.657} = 0.227.$$

Adjusting for the effects of marking as above, the survival rate during the first 2 years of ocean residence of unmarked 3-freshwater Karluk sockeye is estimated to be 31.3%. Using 25.33 months as the duration of the first 2 years of ocean residence of 3-freshwater Karluk sockeye (table 2 of Fredin, MS, 1963), the average monthly instantaneous mortality rate during this period is estimated to be 0.046, approximately 50% greater than the average monthly instantaneous mortality rate during the third year of ocean life. The difference between the average monthly instantaneous rates indicates a temporal decrease in the oceanic mortality rate of salmon analogous to that simulated by Population Model 2 of Fredin (MS, 1963).

DISCUSSION OF METHODS AND ESTIMATES

Maturity Schedule Method

In deriving the equations for estimating an ocean mortality rate from data on maturity schedules and returns, the assumption is made that the survival rate during the last 2 months of ocean life is the same for 2- and 3-ocean fish of a given freshwater age group. Although this seems to be a reasonably acceptable assumption as it stands, its validity should be examined. In this regard, I have obtained some data from the Fisheries Research Institute, University of Washington, on the relative numbers of recoveries in Bristol Bay of 2- and 3-ocean sockeye that were tagged on the high seas 1–2 months prior to their return to the Bay in 1961 and 1963. The release and recovery data are as follows:

Year	Area of tagging	No. of tagged fish released		No. of tagged fish recovered		Per cent recovered	
		2-ocean	*3-ocean*	*2-ocean*	*3-ocean*	*2-ocean*	*3-ocean*
1961	160° W to 180°	1908	2071	358	320	18.8	15.5
1963	175° W to 180°	151	139	11	8	7.3	5.8

Approximately one-half of the tagged sockeye released in 1961 were fish that had been captured in three purse seine sets. The other half was taken in 39 purse seine sets. Observations made during several years of tagging on the high seas, supported by data on recovery rates, indicate that when large numbers of sockeye are taken in a single purse seine set, tagged and released, the tagged fish suffer a greater mortality due to crowding and handling than when smaller numbers of fish are taken in a set.

Ignoring the releases and recoveries of the large number of tagged sockeye that were taken in three purse seine sets in 1961, the percentages recovered in Bristol Bay are as follows:

$$\text{2-ocean: } 227/992 = 22.9\%$$
$$\text{3-ocean: } 270/1090 = 24.8\%.$$

Judging from the foregoing data on releases and recoveries of tagged 2- and 3-ocean sockeye, the survival rate during the last 2 months of ocean life appears to be about the same for 2- and 3-ocean sockeye.

The Bristol Bay runs of 1.485 million 5.3 sockeye in 1958 and 0.787 million 6.3 sockeye in 1959 probably do not represent the total returns of these two age groups, since the Japanese gill net fishery took thousands of 5.3 and 6.3 sockeye in 1958 and 1959, respectively, in high seas areas where Bristol Bay sockeye occur. Table III shows the estimated catches of 5.3 and 6.3 sockeye by the Japanese gill net fishery to have been 0.076 and 0.184 million fish in 1958 and 1959, respectively, between 170° E and 175° E in May, between 175° E and 180° in May and June, and between 180° and 175° W in June and July. The

numbers of 5.3 and 6.3 Bristol Bay sockeye in the high seas catches are not known. Based on the ratios of high seas catches to the numbers of 5.3 and 6.3 sockeye in the Bristol Bay runs (0.076/1.485 = 0.05, and 0.184/0.787 = 0.23), it can be surmised that the Japanese fishery took a larger proportion of 6.3 Bristol Bay sockeye in 1959 than of 5.3 Bristol Bay sockeye in 1958. If this were the case, or if Bristol Bay sockeye accounted for the same proportions of the high seas catches of 5.3 and 6.3 fish in 1958 and 1959, respectively, the estimate of s_{t3} (0.711) would be too low.

The magnitude and direction of error of the proportion of 2-ocean Bristol Bay sockeye assumed to mature in 1958 are not known. If more than 57.3% of the 2-ocean fish returned to Bristol Bay in 1958, the estimate of s_{t3} would be low. If less than 57.3% of the 2-ocean fish returned to Bristol Bay in 1958, the estimate of s_{t3} would be high. For example, if 62.3% of the 2-ocean Bristol Bay sockeye matured in 1958, the estimate of s_{t3} would be 0.876, and the corresponding average monthly instantaneous ocean mortality rate would be about 0.011. If 52.3% of the 2-ocean Bristol Bay sockeye matured in 1958, the estimate of s_{t3} would be 0.581, and the corresponding average monthly instantaneous ocean mortality rate would be about 0.045.

Ocean Immatures Method

In deriving the equation for estimating an ocean mortality rate from data on indices of abundance of immature 1- and 2-ocean sockeye at sea in 1 year and returns of 2- and 3-ocean fish in the following year, the assumption is made that the survival rate during the last 11 months of ocean life is the same for sockeye that mature as 2- and 3-ocean fish in a given year. Although this assumption seems also to be reasonably acceptable as it stands, its validity will be examined as additional data are compiled on the recoveries of mature 2- and 3-ocean sockeye that were tagged on the high seas as immature 1- and 2-ocean fish.

Concerning the usage of indices of abundance of immature 1- and 2-ocean sockeye south of Adak Island to represent the relative numbers of immature 1- and 2-ocean Bristol Bay sockeye at sea on August 1, a possible source of bias is the selectivity of purse seines for immature 1- or 2-ocean sockeye. In this regard, some unpublished data from joint fishing operations (involving purse seines, gill nets, and longlines) south of Adak Island in the summer of 1959 indicate that purse seines are selective for small (immature 1-ocean) sockeye (Fig. 2). If this is generally the case, the ratio of the average catch per seine set of immature 1-ocean sockeye to the average catch per seine set of immature 2-ocean sockeye would generally be greater than the ratio of immature 1- to 2-ocean sockeye in the high seas south of Adak Island, and also probably greater than the relative number of immature 1- and 2-ocean Bristol Bay sockeye at sea. In this event, the estimates of the proportion of Bristol Bay sockeye that mature as 2-ocean fish (equation 13), and s_o, the survival rate during the penultimate year of life of 3-ocean Bristol Bay sockeye (equation 12), would be low.

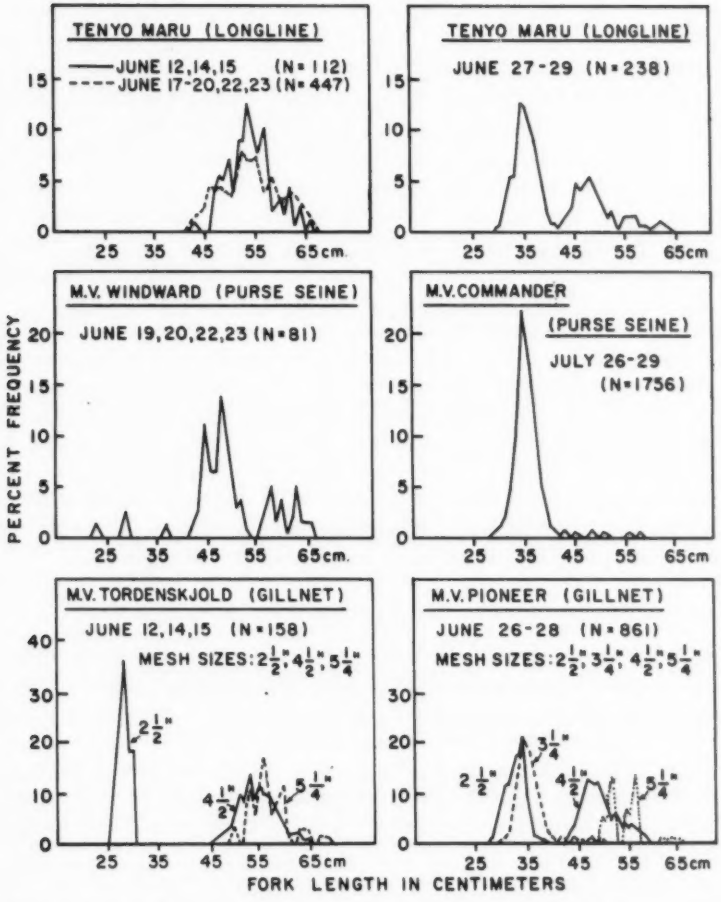

FIG. 2. Length frequency distributions of sockeye caught by longlines, purse seines and gill nets south of Adak Island in summer of 1959. (Unpublished data from Fisheries Agency of Japan, Fisheries Research Institute, University of Washington, and Bureau of Commercial Fisheries, compiled by R. H. Lander and R. R. French.)

The estimated catches of Bristol Bay sockeye by the high seas gill net fishery, hence the estimated total returns of 2- and 3-ocean Bristol Bay sockeye, cannot be considered to be precise. More detailed information on the age composition of the high seas catches would be useful for more accurate statistics on total returns.

Smolt Indices Method

The principal assumptions carried in this method concern constant ocean survival conditions for successive smolt migrations during the first 2 years of ocean residence as well as during the third year of ocean life of 3-ocean fish. That such survival conditions do not generally prevail is shown by estimates of ocean mortality rates of Pacific salmon given by Ricker (1962), Parker (1962), and Fredin (MS, 1963). The variability in the regression of returns of 3-ocean Karluk sockeye per smolt index on returns of 2-ocean fish per smolt index also indicates that survival conditions during the first two and third years of ocean life are not the same for successive smolt migrations. (For example, the expected value for R_{3z}/I_z from the equation, $R_{3z}/I_z = 1.494 - 0.657\ R_{2z}/I_z$, is 0.72 when $R_{2z}/I_z = 1.18$, as compared with the observed value of 0.49).

Although ocean survival conditions vary somewhat for different smolt migrations, and returns of 1- and 4-ocean fish were ignored in the analysis, the estimates of survival rates during the first two and third years of ocean life obtained by the smolt indices method are similar to those obtained under Population Model 2 of Fredin (table 9, MS, 1963) for 3-freshwater Karluk sockeye.

As far as smolt migrations are concerned, it might be no more difficult to estimate total numbers of seaward migrants than to determine the efficiency of a sampling device for obtaining indices of abundance of smolts.

Comparison of Estimates

For 3-freshwater, 3-ocean (6.3) Bristol Bay sockeye that matured in 1959, the natural mortality rate during the year immediately preceding the last 2 months of ocean life is estimated to be 28.9%. This rate corresponds to an average monthly instantaneous mortality rate of 0.028.

The average annual ocean mortality rate during the penultimate year of life of 3-ocean Bristol Bay sockeye for the years, 1956–57 to 1960–61, is estimated to be 19.5%. This rate corresponds to an average monthly instantaneous mortality rate of 0.018.

From data on the returns of six groups of sockeye marked as smolts in the late 1920's and early 1930's, the natural mortality rate during the third year of ocean life of unmarked 3-freshwater Karluk fish is estimated to be 28.2%. This rate corresponds to an estimated average monthly instantaneous mortality rate of above 0.031.

Although the foregoing estimates pertain to different populations, age groups, and periods of time, and are based on different types of data and assumptions, they are similar to one another. The average of the three estimates of annual mortality rates is 25.5%, which is close to the estimate of 27% given by Parker (1962) for the annual ocean mortality rate of Pacific salmon.

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Effect of Polyphosphates and Other Salts on Drip Loss and Oxidative Rancidity of Frozen Fish[1]

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ABSTRACT

Pretreatment of fresh fillets of Dover sole (*Microstomus pacificus*), Pacific cod (*Gadus macrocephalus*), halibut (*Hippoglossus stenolepis*), and red snapper (*Sebastodes ruberrimus*) with sodium tripolyphosphate prior to freezing was found to be effective in reducing drip loss during thawing. Sodium chloride, in a few trials, was effective in reducing drip and cooking losses. Tripolyphosphate and sodium citrate dip solutions of corresponding ionic strengths were equally effective in reducing drip loss during thawing from fillets of red snapper. Pretreatment of chinook salmon (*Oncorhynchus tshawytscha*) fillets with sodium tripolyphosphate preceding freezer storage was found ineffective in retarding lipid oxidation during storage at −10°C; however, unlike sodium chloride, sodium tripolyphosphate did not accelerate development of oxidative rancidity.

INTRODUCTION

Numerous investigators have reported varying results on the use of polyphosphates and salts of weak acids in foods for the purpose of binding moisture within protein constituents. As reported by Taylor (1958) the effectiveness of a polyphosphate must be thought of in statistical terms; individual trials will, in fact, not normally give identical results, but an effective additive may be expected to show an average improvement over a reasonable number of trials. It was stated that a good deal of confusion and disappointment may be avoided if this fact is given consideration.

Hamm (1960) reviewed the subject of biochemistry of meat hydration. Bendall (1954, 1958) reported on the effect of polyphosphates on the contractile protein actomyosin of meats, and on the resultant effect of such a reaction on the water-binding capacity of meat.

Tarr (1942) reported that the prevention of free drip by lightly brining unfrozen fish muscle at its natural pH is brought about by the ability of sodium chloride to cause the proteins to bind liquid firmly. Heen (1954) reported a pronounced difference in the amount of free-water in "brei" from cod muscle with the use of sodium hexametaphosphate. The loss of free drip by thawed haddock fillets may be minimized by incorporation of small quantities of salt into fillets prior to freezing (Holston and Pottinger, 1955). Buttkus and Tarr (1962) found that pre-slaughter intraperitoneal injections of hexametaphosphate into lingcod was effective in reducing drip formation. Thaw drip from frozen

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haddock fillets was decreased by pretreatment of fresh fish with sodium tripolyphosphate at a concentration of 12.5% in dip solutions (Mahon, 1962). Tanikawa *et al.* (1963) reported that polyphosphates were effective in reducing the amount of drip from frozen cod during thawing. Dyer *et al.* (1964) found no differences occurring on storage of cod fillets at −12°C, in extractable protein, lipid hydrolysis, or thaw drip from tripolyphosphate-treated and non-treated control fillets; the low concentration of tripolyphosphate in dip solutions was reported to have no specific effect on thaw drip from frozen cod. MacCallum *et al.* (1964) reported that tripolyphosphate treatment of once- or twice-frozen cod may reduce drip in some instances.

This paper presents the results of five separate experiments which were initiated at the request of the fishing industry, mainly to evaluate the effect of sodium tripolyphosphate on solution uptake and attendant weight increase of fresh fillets during processing and on thaw-drip loss from frozen fillets during thawing of five varieties of Pacific coast marine fish; comparative tests were carried out with various other salts. The possibility that sodium tripolyphosphate could retard lipid oxidation was also investigated in conjunction with drip loss trials on frozen chinook salmon.

MATERIALS AND METHODS

Fresh Pacific coast marine fish were obtained dressed, filleted, or portioned from commercial fish plants; the five varieties used were as follows: Dover sole (*Microstomus pacificus*), Pacific cod (*Gadus macrocephalus*), halibut (*Hippoglossus stenolepis*), red snapper (*Sebastodes ruberrimus*), and chinook salmon (*Oncorhynchus tshawytscha*). The maximum post-mortem age of fish used was 3 days with the one exception that the post-mortem age of halibut was 10 days.

Dip Process

Fillets or portions of fresh fish were immersed for 1 min, unless stated otherwise, in a solution containing the compound under test. Temperatures of dipping solutions varied from 16°C in one commercial trial to 10–14°C in laboratory trials. Subsequent to immersion, treated samples were allowed to drain on inclined aluminum racks for 2–3 min before packaging and freezing. Fish samples were packaged in 1-lb cartons for commercial trials and in polyethylene bags for laboratory trials. Solution uptake was determined by weighing each individual sample on a Toppan 500-g capacity balance (sensitivity ±0.02 g) prior to treatment and again subsequent to draining.

Drip Loss

The term "per cent drip loss" as used throughout this paper refers to the per cent by weight of "free drip", which is the fluid (thaw drip or drip loss) that separates freely during thawing from the frozen product without application of mechanical or manual pressure. After a preliminary examination of several available methods of determining drip loss, the method adopted was a

technique described in a United States Standards publication (Anon., 1960) for grading of frozen cod fillets. In the unmodified original method, a corrosion-resistant metal rod was placed in a moisture-impermeable bag with each frozen test sample for the purpose of immersing the sample in a water bath for drip-loss determinations; circular sieves were used for drained weight determinations. The alteration of technique in the modified method eliminated use of metal rods and circular sieves; frozen fillets or portions of frozen fillet blocks were weighed subsequent to removal of surface ice and extraneous particles of fish. After weighing, each frozen sample was placed in a moisture-impermeable polyethylene bag. To ensure complete moisture impermeability, bagged samples were placed in a larger polyethylene bag which was then immersed in an agitated water bath set at a temperature of 20°C ($\pm$1°C); open ends of large master bags were taped to the top edge of the bath. A fibreglass sheet in which holes were bored was used in conjunction with added weights to immerse bagged samples in the water; beakers were inserted between the fibreglass sheet and bottom plate of the water bath to prevent pressure being applied to samples during thawing. Subsequent to thawing, samples were removed from polyethylene bags, allowed to drain for 3 min on an inclined aluminum rack, and then weighed to determine drip loss.

Two additional methods were used to ascertain the effect of extended thawing periods on drip loss; the methods are described under the sections on Dover sole and Pacific cod.

Cooking Loss

In some experiments, cooking losses were determined on either frozen or thawed fish; the cooking method used in the determination of cooking losses was that of oven-baking as recommended by the Department of Fisheries of Canada (Anon., 1962) with the following alterations: (1) additives such as oil were not used, and (2) fish were baked on racks covered with perforated aluminum foil to allow fluids to drain. Subsequent to cooking, samples were allowed to cool at room temperature for approximately 15 min prior to weighing for cooking loss determinations.

Oxidative Rancidity

To ascertain the degree of oxidative rancidity, the method of Tarr (1947) was used, with a few minor modifications, to determine peroxide values (PV). Fine shavings cut from frozen fillets were homogenized in a Servall Omnimixer; the homogenate was used for PV determinations. Duplicate samples were used from each treatment.

Texture

A brief investigation was carried out in conjunction with drip loss studies to evaluate the effect of polyphosphates on the shear force required to cut muscle fibres of cooked fish muscle subsequent to storage of fresh fillets at

–20°C. The shear force required to cut fibres of fish muscle was measured using a modified shear apparatus (Gadd and Buttkus, 1964).

Application of Methods to Various Fish

DOVER SOLE

Trial I. Fresh skinless sole fillets were processed at a fish plant under commercial conditions and immersed for 2 min in one of the following solutions: water as a control; sodium tripolyphosphate (TPP) 8% (W/V); sodium hexametaphosphate (HMP) 10%. The temperature of dip solutions was 16°C. Approximately 20 lb of fillets were used for each treatment; on the average there were 3–4 fillets per pound. Fillets were packaged in 1-lb cartons, frozen initially in a commercial plate freezer, packed in master cartons, and then stored at either –10 or –20°C in laboratory freezers. One-pound cartons of frozen fillets were removed from storage at intervals and were cut with a saw to provide portions of 200–250 g which were used for drip loss determinations. After 1 month storage at –10°C, three frozen fillets from each treatment were initially thawed for 6 hr on an inclined aluminum rack set in a polyethylene container; the relative humidity (RH) and temperature during thawing were 70% and 20°C, respectively. During an extended thawing period of 64 hr at 0°C the container was covered with aluminum foil to prevent moisture loss; at intervals the fillets were weighed to ascertain the amount of drip loss. After 7 months storage at –20°C, 16 frozen fillet portions from each treatment were thawed in a water bath for periods of 1½–3 hr (Exp. 1); after 9 months storage at –20°C, six portions from each treatment were thawed in a water bath for 2 hr (Exp. 2) and subsequently oven-baked.

Trial II. Fresh sole fillets, six skinless and six non-skinned with an approximate weight each of 175 g, were immersed in each of the following solutions: water as control; potassium citrate (KCit), 15%; sodium citrate (NaCit), 15%; sodium chloride (NaCl), 4%; TPP, 12.5% (Mahon, 1962). Twelve fillets which were not immersed in solution were used as non-dipped controls. After 3 weeks storage at –20°C, the fillets were removed from storage and thawed for 1½ hr by the water bath method.

Trial III. Ten skinless fresh sole fillets, with an approximate weight of 150 g each, were immersed in each of the following solutions: water as control; TPP 12.5%; KCit, 10, 15, and 20% concentrations. Subsequent to storage at –20°C for 2 weeks, frozen fillets were removed from storage and thawed for 45 min by the water bath method.

PACIFIC COD

Trial I. To evaluate the effect of NaCl on weight gain and on drip loss, six fresh Pacific cod fillets with an approximate weight each of 150 g were immersed in each of the following NaCl solutions: 0, 4, 8, 10, 12, and 15%. After 5 days storage at –20°C, frozen fillets were removed from storage and thawed for 1½ hr by the water bath method.

Trial II. Fillets of Pacific cod with an average weight of 150 g were immersed in each of the following solutions: water as control; NaCit, 15%; TPP, 12.5%; 12–14 fillets were used for each treatment. After storage for 30 days at −10°C (Exp. 1), four fillets from each treatment were allowed to thaw initially for 5 hr at 18°C and 70% RH and then for an additional 60 hr at 0°C. After 3 months storage at −10°C (Exp. 2), six fillets from each treatment were thawed initially for 1 hr by the water bath method and then for an additional 16 hr at 0°C.

In an additional procedure with Trial I fish (Exp. 3), the method used for thaw drip determinations consisted of placing weighed small cut portions of frozen fish in a funnel, covering the funnel top with aluminum foil, collecting drip in a test tube or graduated cylinder during thawing and then determining drip loss by weighing drip and also by weighing thawed flesh. Thawing was done at room temperature for 2 hr and then for an additional 18 hr at 0°C; six samples were used for each treatment.

HALIBUT

Portions of halibut weighing approximately 120 g each were immersed in each of the following solutions: water as control; TPP, 12.5%; NaCl, 15%; KCit, 15%; NaCit, 15%; 15 portions of halibut were used for each treatment. Non-dipped portions of halibut were also packed and stored for use as controls. After storage for a 2-week period at −20°C, the portions were removed from storage and thawed for 1 hr by the water bath method.

SALMON

In each of two experiments, large fresh chinook salmon were obtained from a fishing vessel on the day of catching; fillets weighing approximately 400 g were cut from the fish. Fillets from an individual fish were immersed in each of the solutions under test for the purpose of obtaining representative samples and making tests comparable.

Trial I. Fresh fillets were immersed in each of the following solutions: water as control; NaCl, 8%; TPP, 8%; HMP, 8%. Samples were stored at −10° or −30°C; at intervals fillets of each respective treatment were removed from storage and thawed under varying conditions; 10 fillets from each treatment were used in drip-loss tests. Subsequent to thawing, fillets were cooked to assess total weight loss.

Trial II. In this trial, fillets were immersed in each of the following solutions: water as control; TPP, 12.5%; NaCit, 15%; for each treatment 18 fillets were processed for use in determination of solution uptake, drip loss, cooking loss, and oxidative rancidity. Subsequent to storage for 2 months at −10°C, four fillets from each treatment were thawed for 1½ hr by the water bath method, and then cooked to determine total weight loss.

After 150 days at −10°C, samples were removed from storage to determine the extent of oxidative rancidity.

RED SNAPPER

To ascertain the effect of ionic strength of dipping solutions on the resultant water-holding capacity of frozen red snapper, fillets with an approximate weight each of 120 g were immersed in each of the following solutions: water as control; TPP, 12.5%; NaCit, 15 and 25%; 15–30 fillets were used for each treatment. Subsequent to storage at −20°C for 21 days, fillets were removed from storage and thawed for 1 hr by the water bath method.

RESULTS AND DISCUSSION

Influence of pH of Dip Solutions on the Resultant pH of Muscle Sample

Investigators have reported the various effects of pH on the water-holding capacity of fresh and heated meat. Thus with fish muscle it was shown that swelling was increased very markedly above, and depressed strongly below, the isoelectric zone by addition of NaOH and HCl, respectively (Tarr, 1942). Hellendoorn (1962) studied the action of different phosphate salts on water binding of ground meat at various pHs; at pH values below 5.5, pyrophosphate and

Table I. Effect of polyphosphates and salts on the pH of dip solutions and of treated fish portions.

Dip solutions[a]	Water	TPP		NaCit		KCit		NaCl		HMP	Not dipped
Concentration (%)		8	12.5	15.0	25.0	15.0	20.00	8.0	15.0	8.0	
Test sample	pH										
Salmon											
(1) Dip soln.	6.4	9.0						6.2		5.8	
Flesh	6.3	6.3						6.2		6.2	
(2) Dip soln.	6.3		9.2	7.5							
Flesh	5.7		5.9	5.8							
Sole											
(1) Dip soln.	6.2		9.0	7.4		7.5					
Flesh	6.2		6.3	6.3		6.4					6.2
(2) Dip soln.	6.2		8.7				7.2				
Flesh	6.5		6.6				6.6				
Red snapper											
Dip soln.	6.9		9.0	7.5	7.5						
Flesh	6.7		6.9	6.7	6.8						
Halibut											
Dip soln.	6.3		9.0	7.5		7.6			6.9		
Flesh	5.9		6.1	6.1		6.1			6.1		6.0

[a]TPP = sodium tripolyphosphate; NaCit = sodium citrate; KCit = potassium citrate; NaCl = sodium chloride; HMP = sodium hexametaphosphate.

tripolyphosphate exerted a depressing effect on the water binding of uncooked meat. Ohta and Nishimoto (1963) reported that TPP was effective in reducing centrifuge drip of minced mackerel flesh; pH was found to influence the extent of drip of minced flesh. It was found that adjusting the pH of minced mackerel flesh with dilute alkali to a pH value corresponding to that of polyphosphate treated flesh was not as effective as sodium tripolyphosphate in reducing drip.

Since there was a difference in pH values of dip solutions employed in the present investigation, it was considered important to ascertain the pH of treated portions of fish; pH values were determined on homogenized muscle. The results are shown in Table I. In all tests it may be noted that there is no significant difference in the pH values of fish flesh within a single test series.

Dover Sole

In Fig. 1 and 2 the results of tests which were carried out on sole fillets in Trial I are recorded. Although the concentration of TPP (8%) used in the dip solution for this trial was not the concentration of TPP (12.5%) that was found most effective in reducing drip loss from frozen fish by Mahon (1962), it is evident from the results that drip loss was significantly reduced with use of polyphosphates. Pretreatment of sole fillets with polyphosphates was effective in reducing the amount of weight lost during thawing and cooking. The results from Trial II on sole are graphically presented in Fig. 3. Under the conditions used in this trial, the immersion of fillets in a solution of TPP, KCit, or NaCl prior to freezing resulted in an increase of solution uptake when compared with the solution uptake by water-dipped control fillets. The solution uptake results of NaCl-treated samples are in agreement with results obtained by Tarr (1942),

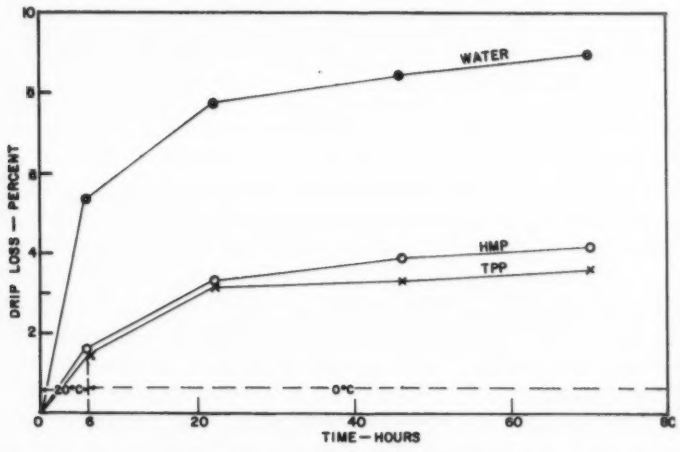

FIG. 1. Influence of TPP and HMP on thaw drip of frozen sole fillets stored at −10°C for 1 month. Frozen fillets were thawed initially at room temperature and subsequently at 0°C.

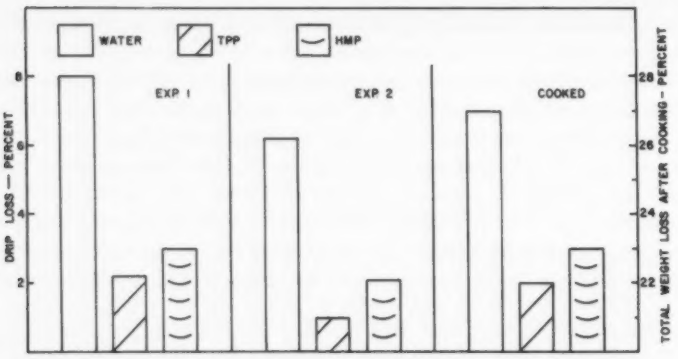

FIG. 2. Effect of TPP and HMP on thaw drip and cooking loss of sole fillets after storage at −20°C for 7 months (Exp. 1) and 9 months (Exp. 2). Thawed fillets from Exp. 2 were used in cooking loss tests.

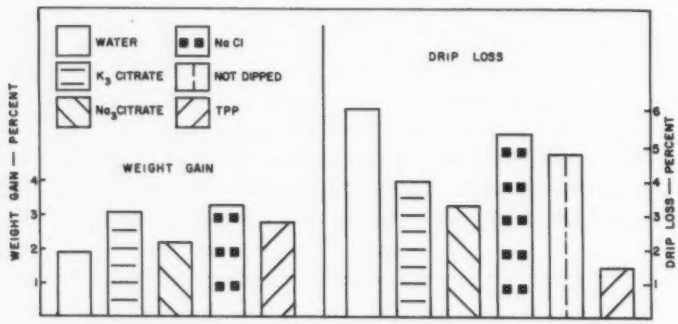

FIG. 3. Influence of TPP, citrates, and NaCl on solution-uptake and thaw drip of sole fillets. Frozen fillets were removed from storage after 3 weeks at −20°C.

Holston and Pottinger (1955), and Mahon (1962) from studies on the effect of pretreatment of fish with NaCl. It may be noted that drip loss from non-dipped control fillets was slightly less than drip loss from water-dipped control fillets. In this trial, TPP was more effective in reducing the amount of thaw drip from frozen fillets during thawing than citrates and NaCl. Although the low concentration of NaCl (4%) in dip solutions was not effective in reducing thaw drip, it was thought that such a concentration might have an effect on thaw drip. Holston and Pottinger (1955) found in some instances that immersion of fillets for 1 and 2 min in a 5% NaCl solution resulted in a salt concentration in fillets of 0.67 and 0.98%, respectively; they also reported that in some instances free drip from certain varieties of fish was reduced by NaCl concentrations in the muscle as low as 0.58%.

TABLE II. Influence of TPP and KCit on thaw drip and solution uptake of sole fillets. Fillets stored at −20°C for 14 days (Trial III).

Dip Solution	Weight gain (%)	Drip loss (%)
Water	1.4	4.4
KCit 10%	1.8	3.3
15%	1.9	3.3
20%	1.8	2.7
TPP 12.5%	2.5	2.1

Table II shows the results which were obtained from Trial III on sole fillets. TPP-treated fillets picked up more solution than fillets from other treatments. When consideration is given to differences between solution uptake and thaw drip of treated fillets, it will be noted that a positive yield of product was obtained only from TPP-treated fillets. Solution uptake was greater in treated fillets than in untreated fillets; thaw drip was less from treated fillets than from untreated fillets. Dyer *et al.* (1964) reported that treatment of cod fillets for freezing increases the yield of the frozen and thawed product.

Tarr (1942), Bendall (1954), Hellendoorn (1962), and Dyer *et al.* (1964) reported on the significance of actual salt concentration (ionic strength) within treated fish or meat products on swelling, moisture retention, and thaw drip of various tissues. Since the present report was initiated mainly to evaluate the efficacy of TPP at various concentrations and at a concentration recommended for use with fish muscle (Mahon, 1962) in reducing thaw drip, the determination of amount of salts absorbed by fish samples during treatment was not included in experimental trials; Mahon (1962) reported on the absorption of polyphosphates by fish flesh.

From results of a limited investigation which was carried out to ascertain the effect of polyphosphates on "toughness" quality of baked sole, it was found that less shear force was required to shear fibres of TPP treated fillets than was required to shear fibres of control samples. Shear-force measurements on sole fillets were found to be tedious with use of a modified shear apparatus (Gadd and Buttkus, 1964) because of the inherent fine, short muscle fibres of sole. Shear-force measurements do not necessarily indicate an increase in organoleptic toughness (Gadd and Buttkus, 1964).

PACIFIC COD

In Fig. 4 the results of Trial I on Pacific cod fillets are presented. The results reveal that within the range used, NaCl concentration in dip solutions did have a slight effect on solution uptake. The maximum amount of solution uptake occurred at the 12% NaCl level, which is in agreement with results obtained

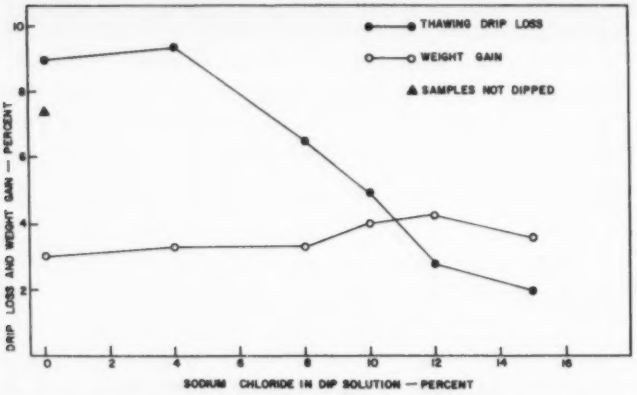
THAWING DRIP LOSS
WEIGHT GAIN
SAMPLES NOT DIPPED
DRIP LOSS AND WEIGHT GAIN — PERCENT
SODIUM CHLORIDE IN DIP SOLUTION — PERCENT

FIG. 4. Influence of NaCl on solution uptake and thaw drip of Pacific cod fillets. Fillets were stored at −20°C for 5 days.

by Holston and Pottinger (1955) who reported a maximum uptake of solution at 10–15% NaCl in dip solutions. The per cent drip loss from water-dipped control fillets and non-dipped control fillets was 9.0 and 7.5%, respectively. At a concentration of approximately 11% NaCl in dip solutions, the solution uptake and thaw drip appear to equilibrate.

Table III shows the results of Trial II on Pacific cod fillets. The yield of Pacific cod fillets was increased by immersion in TPP and NaCit solutions. In Exp. 1 after an extended thawing period, TPP was effective in reducing the amount of thaw drip; in Exp. 2 results obtained were not comparable with those from Exp. 1. The amount of thaw drip from treated samples in the two experiments was comparable, but thaw drip from the control samples was not comparable; in Exp. 2 the water-dipped controls might have lost considerable moisture during the additional 2 months storage at −10°C, and thus the amount of moisture lost during thawing would be less than from a non-desiccated sample.

TABLE III. Influence of TPP and NaCit on thaw drip and solution uptake of Pacific cod fillets

Dip solution	Weight gain (%)	Drip loss (%)		
		Exp. 1[a]	*Exp. 2*[b]	*Exp. 3*[c]
Water	2.5	4.2	1.8	9.0
NaCit 15%	3.2	2.0	2.2	4.0
TPP 12.5%	3.9	1.0	1.6	4.2

[a]Frozen fillets stored for 30 days at −10°C.
[b]Frozen fillets stored for 3 months at −10°C.
[c]Frozen fillets stored for 2 months at −10°C. Fillets were cut into small portions, placed in a funnel and thaw drip collected in a graduated cylinder.

In Exp. 3 (Table III) where thaw drip was determined on small cut portions of fish, it will be noted that from a comparative standpoint TPP and NaCit were effective in reducing thaw drip.

Halibut

The results obtained from experiments on halibut are presented in Fig. 5. Although the weight gain of halibut portions from each treatment was not excessive or variable, portions immersed in the NaCl solution picked up a greater percentage of solution than did portions from the remaining treatments; the increase in yield of product from each treatment is of significance. In examining per cent drip loss of water-dipped controls and non-dipped portions, it is evident that there is no significant difference in the results. All treatments were effective in reducing thaw drip.

Salmon

Although thaw drip and solution uptake (weight gain) of salmon flesh during processing are not considered important factors, two separate trials were carried out on chinook salmon to ascertain the effect of TPP, HMP, NaCl, and NaCit on drip loss, solution uptake, and cooking loss. Table IV shows the results obtained from Trial I on salmon. None of the compounds had any appreciable effect on drip loss from frozen salmon flesh. The compounds were effective in reducing the amount of material lost during cooking. The results obtained from Trial II on salmon are presented in Fig. 6. There was a slight increase in solution uptake of TPP and NaCit treated fillets; drip and cooking losses of TPP and NaCit treated fillets were comparable to losses of water-dipped controls.

A brief investigation was carried out in conjunction with drip loss studies to evaluate the effect of polyphosphates on the shear force required to cut

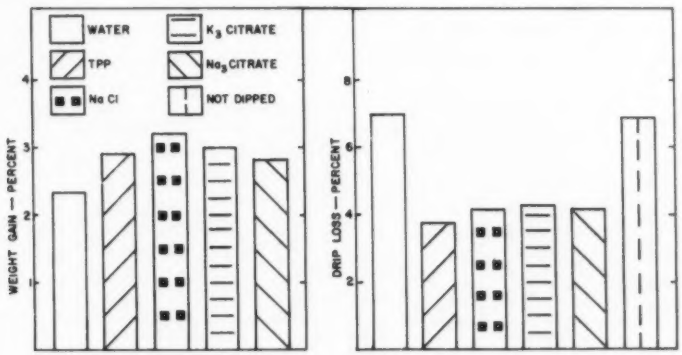

Fig. 5. Influence of TPP, citrates, and NaCl on solution uptake and thaw drip of halibut. Samples were stored at −20°C for 3 weeks.

TABLE IV. Effect of TPP, HMP, and NaCl on solution uptake and thaw drip of salmon fillets.

Dip solution	Weight gain (%)	Drip Loss[a] (%)	Total weight loss after cooking (%)
Water	1.4	1.7	17.0
TPP 8%	3.2	1.5	15.8
HMP 8%	2.5	1.6	15.7
NaCl 8%	2.5	1.6	14.5

[a]Drip loss results represent a summary of tests which were made on frozen fillets after storage for 3 and 4 months at either −10° or −30°C.

muscle fibres of cooked salmon muscle. After 5 months storage at −20°C, shear tests on baked salmon fillets revealed that 23% less force was required to shear fibres of TPP-treated fillets than was required to shear fibres of control samples.

INFLUENCE OF TPP ON RANCIDITY DEVELOPMENT

Ramsey and Watts (1963) reported that TPP, when added to ground mullet and beef, decreased lipid oxidation with increasing concentrations of TPP in the product; it was reported that the mechanism of polyphosphate activity is unknown. Tims and Watts (1958) reported that polyphosphates, with the exception of orthophosphate, retarded oxidative rancidity in meats; ascorbic acid acted synergistically with phosphates.

The results of four tests which were carried out to evaluate the effect of TPP and NaCit on the development of oxidative rancidity in frozen chinook

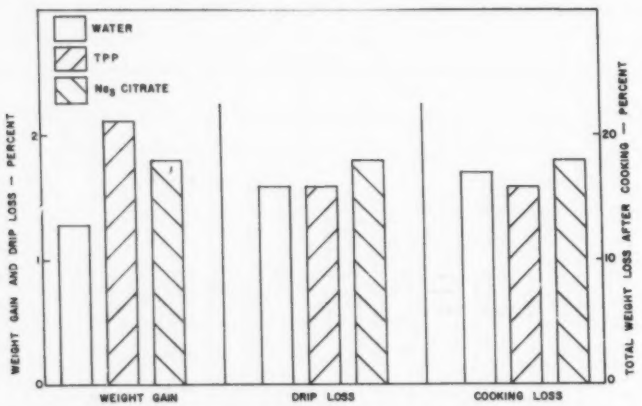

FIG. 6. Effect of TPP and NaCit on solution uptake, thaw drip, and cooking loss of chinook salmon. Frozen fillets were removed from storage after 2 months at −10°C.

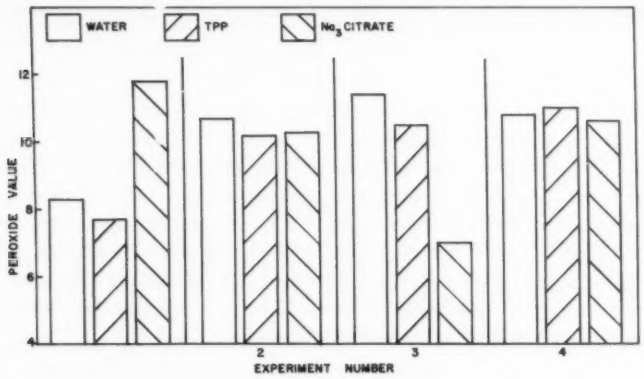

FIG. 7. Influence of TPP and NaCit on rancidity development in frozen chinook salmon fillets. All samples were stored at −10°C for 150 days as in Exp. 1 with subsequent storage at −20°C as follows: Exp. 2 — 30 days, Exp. 3 — 60 days, Exp. 4 — 65 days.

salmon during freezer storage are presented in Fig. 7. The results indicate that under these experimental conditions, TPP was not very effective in retarding development of oxidative rancidity in chinook salmon. Variable results were obtained from NaCit-treated samples. Although it may be stated that the storage conditions were unfavorable for comparative evaluation, Tarr (1946, 1947) reported that ascorbic acid and ethyl gallate were effective antioxidants in retarding development of rancidity in chinook salmon fillets after storage at −10°C for periods up to 120–149 days.

To ascertain the effect of the cooking of fish on the resultant PV index for determining lipid oxidation, chinook salmon fillets were removed from storage after 150 days at −10°C and tested for the degree of lipid oxidation in cooked and uncooked portions of fillets from one fish; six portions were used from each treatment for PV determinations.

The results are shown in Table V. As reported by Tarr (1947), lower PV are obtained when chinook salmon flesh is heated, irrespective of pretreatment.

TABLE V. Peroxide values of cooked and uncooked chinook salmon fillets from same fish pretreated with TPP and NaCit (stored for 150 days at −10°C).

Dip solution	PV-ml 0.002*N* $Na_2S_2O_3$/g of fat	
	Cooked	*Uncooked*
Water	4.3	10.1
TPP 12.5%	3.8	9.8
NaCit 15%	4.5	10.4

Red Snapper

The results of an experiment on red snapper are presented in Fig. 8. The experiment was designed specifically to compare the effect of TPP and NaCit dip solutions of comparable ionic strength on the resultant water-holding capacity of frozen fillets during thawing. The results indicate that the salts under test had no appreciable effect on solution uptake; when drip loss from water-dipped control fillets is compared with drip loss from treated fillets, it will be noted that product yield was increased in fillet samples pretreated with TPP and NaCit (25%). It is evident that the ionic strength of dip solutions has a very significant effect on the moisture holding capacity of fish muscle. The ionic strength of dip solutions will undoubtedly have a very significant effect on the resultant ionic strength of treated fish muscle.

Organoleptic Observations

The results obtained from a limited number of organoleptic tests on baked samples of TPP-treated and untreated sole and salmon fillets indicated, although not consistently, that treated samples had a higher moisture content and a fresher flavour than untreated fillets; organoleptic findings from other tests were not always in agreement with the above results. To evaluate the effect of TPP in preventing or retarding loss of product quality during frozen storage, it would seem advisable to carry out extensive trials under various storage conditions.

CONCLUSION

It is concluded from the results as presented in this paper that pretreatment of fresh fillets of certain varieties of fish with TPP prior to freezing is effective in reducing drip loss during thawing and in increasing product yield of both frozen and thawed fish; comparable results were obtained in some trials with use of NaCl and citrates at reasonably high concentrations. At the concentration

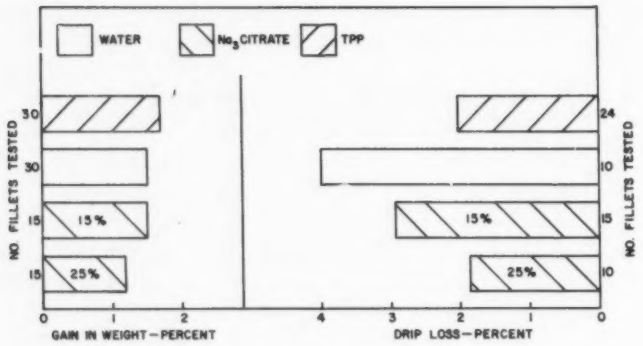

Fig. 8. Influence of TPP and NaCit on solution uptake and thaw drip of red snapper fillets stored at −20°C for 21 days.

of TPP (12.5%) used in dip solutions for the pretreatment of chinook salmon, it was found that TPP was not an effective antioxidant for use in frozen salmon during storage; unlike NaCl, TPP did not act as a pro-oxidant.

Treatment of fresh fillets of certain varieties of fish with TPP prior to freezing would be of value if subsequent improper handling at the consumer level resulted in thawing considerably beyond the defrost stage preceding cooking.

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Larvae and Megalopa of *Argis dentata* (Crustacea: Decapoda) from Ungava Bay[1]

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ABSTRACT

Stage I and II larvae and the megalopa of *Argis dentata* Rathbun are described from plankton taken by the *Calanus* Expedition in Ungava Bay during July–August, 1947–1950.

INTRODUCTION

The CRANGONIDAE of the northwest Atlantic are few in numbers of species and may be easily recognized by their sub-chelate 1st pereiopods. Even in 1st Stage larvae (as taken from Ungava Bay, northern Quebec, in this study) the apposition of the dactyl of the 1st pereiopod is evident. In Stages I–III (at least) the sub-chela is probably not functional.

Gurney (1923) gives the chief characteristics of the family Crangonidae that distinguishes it from the Hippolytidae and Pandalidae as follows. (These three are the most predominant families of shrimps in Ungava Bay.)

a. The carapace is without dorsal or supraorbital spines and its anterior ventral edge is denticulate.
b. The 5th abdominal segment has a pair of lateral spines. Lebour (1931) says that the fourth abdominal segment may also have a pair of lateral spines. These are not present in *Argis*. Williamson (1960) denotes one genus with spines on the third, fourth, and fifth abdominal segments.
c. The scale of the 2nd antenna is not jointed at the end in early stages (in all stages, Lebour, 1931).
d. The basis of the 1st maxilla has no outer seta.

Williamson (1960) gives a key to the larvae of the genera of the Crangonidae from the northern Atlantic. *Argis* is not included since it has not been described before. Using Williamson's key, *Argis* appears in the same category as *Crangon*. To separate *Crangon* from *Argis*, therefore, the required addition to the key would be:

Pleopods are not developed in Stages I or II. Expansion lacking on proximal section of antennule in Stage II.	*Crangon*
Pleopods well developed in Stage I. Expansion present on proximal section of antennule in Stage II.	*Argis*

The megalopa or post-larval stage of *Nectocrangon lar* (= *Argis dentata*) was described and figured by Stephensen (1916). For comparison it is described

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in detail (Fig. 5 and 6) from specimens obtained in Ungava Bay. Williamson (1957) favours the name of "megalopa" to that of the ambiguous term "post-larva."

METHODS

Larval stages of *Argis* from the plankton were identified, and carapace and total lengths were measured. Several specimens of a larval stage were selected and the right and left mouth appendages of each were dissected out and examined for comparison of setal number, shape, etc. Counts were made of setae of each appendage of about 10 larvae. Drawings in pencil were then made of typical appendages, first making a camera lucida sketch for size and proportion and adding details at high magnification. A description of each appendage

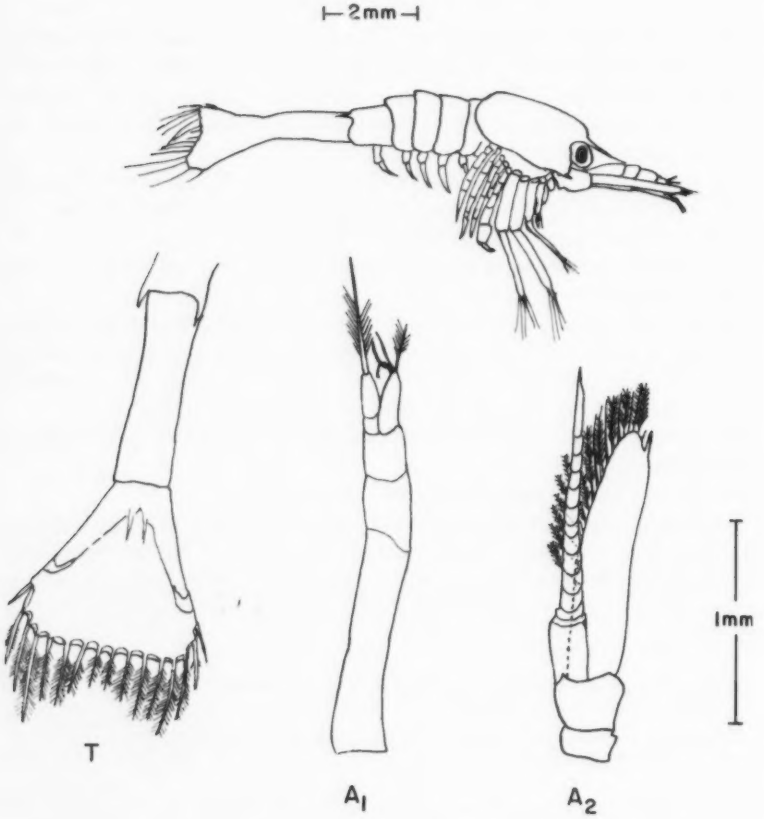

FIG. 1. Stage I larva of *Argis dentata* from Ungava Bay (1.7–2.2 mm in carapace length; 8.0–9.5 in total length). T = telson; A_1 = first antenna (antennule); A_2 = 2nd antenna.

was done from the actual magnified appendage as the pencil sketches were inked in. The outside face of appendages from the left side are shown except when some important morphological detail is present on the inner face only.

STAGE I ZOEA

Total length of this larva is 8–10 mm in specimens from Ungava Bay and the carapace length about 2.0 mm (Fig. 7).

Generally speaking it is an advanced zoea with pereiopods and pleopods well developed. [The characteristic involvement of the eyes (eye-stalks attached to the carapace for their full length) in this Stage I zoea is typical.] No tubercle or supraocular spine is present on the carapace. The rostrum reaches about one-half the length of the antennular peduncle. A pterygostomian spine is prominent followed by 3 equidistant denticles on the anterior edge of the carapace.

The antennules have small flagella, one terminating in a stout plumose seta and the other in 3 projections plus a plumose terminal seta. Their peduncles appear to be 3-sectioned, the first section longer than the other two combined (Fig. 1,A_1). The antennal scale is scarcely exceeded by the sparingly annulate flagellum and has a fan of about 18 plumose setae to two-thirds the length of the scale from the tip. The outside spine slightly exceeds the tip of the scale and it is followed at once by a small spine (Fig. 1,A_2).

The telson (Fig. 1, T) has 8 pairs of terminal setae, the central 7 pairs of which are plumose. The central pair is shorter than the others. A single pair of lateral spines is present on the 3rd abdominal segment.

Mouth Appendages (Fig. 2)

MANDIBLES. Incisor and molar processes are present. The incisor of the left mandible has 3 short teeth while that of the right has 2 teeth. Also between the incisor and molar processes of the left mandible there are generally 3 denticles where the right has but 2 denticles.

FIRST MAXILLA (Mx_1). The endopod is bifurcate distally, the apical with 3–4 and other branch with 2 sparingly plumose setae. On the inner edge the coxa has about 4 long and recurved spiny setae plus 2 short simple setae at each side. The basis has two rows of 6–8 short, stout, and spiny setae, one row of which is half the length of the other.

SECOND MAXILLA (Mx_2). The exopod exceeds the endopod in length and it has a fan of about 4 plumose setae distally followed by 2 divergent setae at its proximal end outside. The endopod has stout plumose setae: 2 apically, and 1, 1, and 2 on each of its other three divisions. The basis and coxa are both bilobed. Distally (on the inside) each lobe has plumose setae comprising about 3 and 4 on the basis and 2 and 8 on the coxa.

FIRST MAXILLIPED (Mp_1). The exopod is more than twice the length of the endopod. It has 3 plumose setae apically plus 1 at the base of the apical projection. The endopod is 4-sectioned, the distal section with 2 simple and 2 plumose

setae (sometimes 4 simple setae), the others 2, 1, and 3 setae, respectively, most of which are plumose. The basis has an inner fringe of about 14 plumose setae in four groups and the coxa about 4 plumose setae singly (sometimes fewer as in Fig. 2).

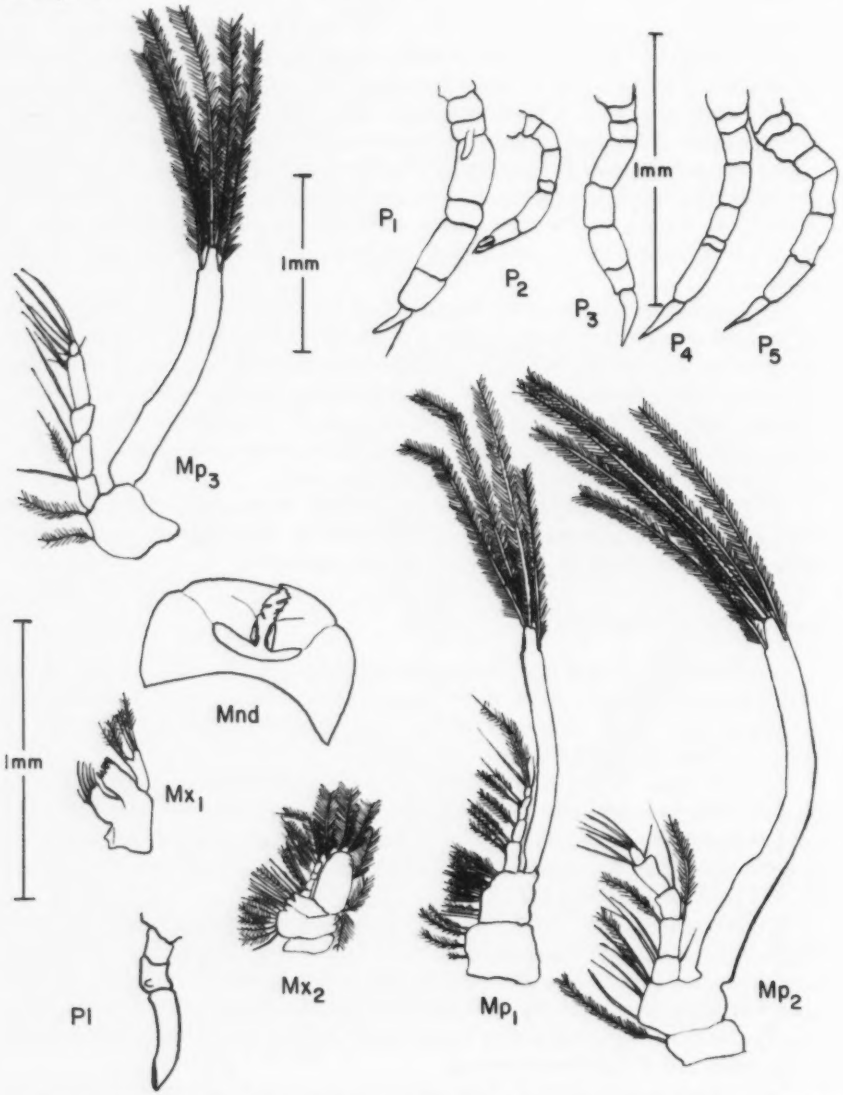

FIG. 2. Stage I larva of *A. dentata*. Appendages: Mnd = mandible; Mx_1 = 1st maxilla; Mx_2 = 2nd maxilla; Mp_1 = 1st maxilliped; Mp_2 = 2nd maxilliped; Mp_3 = 3rd maxilliped; P_1–P_5 = pereiopods 1–5; Pl = 1st pleopod.

SECOND MAXILLIPED (Mp_2). The exopod is almost 3 times as long as the endopod. Its apical projection has 3 long plumose setae at the tip and 1 on each side of the tip. The endopod is 5-sectioned. The apical section has 4 simple setae distally and the other four sections 2, 0, 1, and 3 setae at the inner edge and 1, 1, 1, and 1 setae at the outer edge, respectively, some of which are plumose. The basis has three groups of 3, 2, and 1 setae and there is 1 long plumose seta on the coxa.

THIRD MAXILLIPED (Mp_3). The exopod has 3 long plumose setae apically and 1 on each side of the apical projection. The endopod is 5-sectioned and almost as long as the exopod. The sections each have 3, 3, 1, 1, and 2 setae inside, respectively. There are 2 plumose setae on the basis.

PEREIOPODS (Fig. 2)

FIRST PEREIOPOD (P_1). A rudimentary exopod is present on the basis. The dactyl is fingerlike and suggests the early form of a subchelate claw. A long seta is present beside the insertion of the dactyl on the propodus.

SECOND PEREIOPOD (P_2). The dactyl is about equal to the end of the propodus and with it forms a chela. This pereiopod is shorter than the other pereiopods by about one-third their length.

THIRD TO FIFTH PEREIOPODS (P_3–P_5). These are somewhat similar to each other in appearance and about equal in length.

PLEOPOD (Pl). The coxa, basis, and a large exopod are present. The endopod is seen as a small bud on the basis distally and inside.

STAGE II ZOEA

The moult into Stage II results in an increase in length of about 2.2 mm total length and 0.2 mm carapace length over Stage I (Fig. 7). Differences in morphology from Stage I zoea include an outer expansion or wing formation of the 1st antennal section, as well as the development of a spine below at about two-thirds the length of this section. On the second antenna two inner spines are found on the peduncle at the insertion of the flagellum and the scale. The setal fan of the scale has increased from about 20 setae in Stage I to about 25 setae in Stage II. The setae on the exopods of the maxillipeds have also increased by 1 to 5 rather than 4 in the 1st maxilliped and 6 rather than 5 in the 2nd and 3rd maxillipeds (Fig. 3 and 4).

The carapace has no supraocular spine or median tubercle, but it has a pterygostomian spine and just behind this at the ventral edge anteriorly are 2 minute denticles. The rostrum is medium in length, subequal to the first section of the antennular peduncle and is tapered to a sharp point (Fig. 3).

FIRST ANTENNA (Fig. 3, A_1). It has a peduncle divided into 1 long and 2 short sections. The proximal section has an expansion or wing outside for about one-third its length. Distally and outside 2 medium non-plumose setae are on sections 1 and 2. On the inside below is a curved spine on the 1st section at two-thirds its length distally. The 3rd section bears distally a long plumose seta and

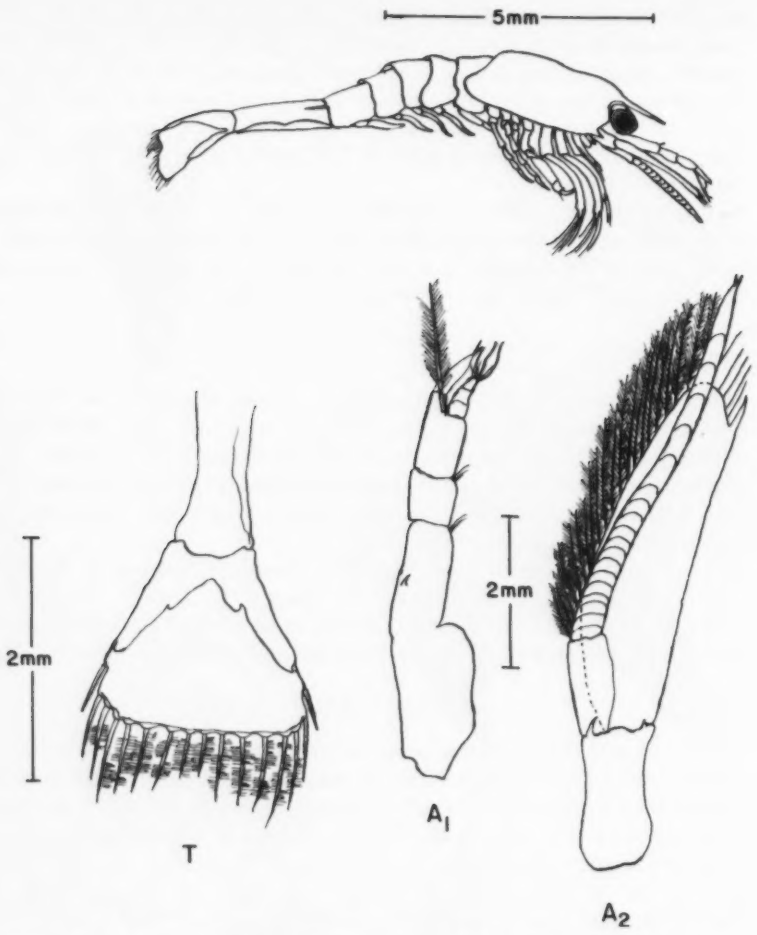

FIG. 3. Stage II larva of *A. dentata* from Ungava Bay (2.0–2.5 mm in carapace length; 9.0–11.0 mm in total length). Lettering is the same as in Fig. 1.

short, developing, flagella. The inner of these flagella has 1 annulation and ends in a slender point divided into 2 at the tip. The outer flagellum has 2 or more annulations and ends in 3 weak slender projections and several fine setae.

SECOND ANTENNA (Fig. 3, A_2). The flagellum now exceeds the scale by about one-fifth its length and it is tipped by a short spine with 2 points. The scale is but slightly notched and has a strong spine at its outer distal edge about equal to the forward edge of the scale. The scale has an evenly-spaced fan of about 26 setae all of which are plumose except near the spine. The peduncle of the flagellum is about one-quarter its length. The antennal peduncle has a

strong spine below at the base of the flagellum and a small one below at the base of the scale.

TELSON (Fig. 3, T). The telson is not appreciably different in Stage II from the telson in Stage I. The abdomen is also similar in both stages.

MOUTH APPENDAGES (Fig. 4)

MANDIBLES (Mnd). The right has an incisor process with 2 short teeth and 3 or 4 long denticles between it and the molar process. The left has an incisor process with 3 or 4 teeth followed by 1 long, 1 short, and 3 long denticles between it and the molar process.

FIRST MAXILLA (Mx_1). This consists of a short endopod, a basis, and a coxa. The endopod is bifurcate; one fork is more terminal than the other and has 3 plumose setae, the other has 2 or sometimes 3 plumose setae. The basis has 2 rows of from 7 to 9 toothlike, short and stout, spiny setae. The coxa has 4 long curved setae with short spiny plumes and a short simple seta at each side of this group of setae.

SECOND MAXILLA (Mx_2). An exopod and endopod as well as the basis and coxa are present. The exopod has 5–8 long plumose setae at its apex plus 3–5 simple setae on each side. Two divergent plumose setae are present at the base of the exopod outside. The endopod is divided into 4 parts each of which has an inner projection bearing 2, 1, 2, and 2 plumose setae. The basis has two subequal lobes with 3–4 and 3–5 plumose setae, respectively. The coxa has a large lobe with 8–10 plumose setae and a small lobe with 2 or 3 (plumes are omitted on some setae in Fig. 2).

FIRST MAXILLIPED (Mp_1). The exopod terminates in 4 long setae (plumose and annulated) and has one sub-terminal seta on the outside. The endopod is 4-sectioned with 3, 1, 1, and 2 setae inside. The basis has 2 irregular rows of about 20 plumose setae on its inner edge (1 row is shown), and the coxa 3 plumose setae.

SECOND MAXILLIPED (Mp_2). The exopod has 6 terminal plumose setae: 4 apical and 1 on each side of the terminal projection. The endopod has a variable number of setae on the apical to 5th sections. These are 5, 1–3, 0, 1, 1–3 inside, and 1–2, 0, 1 outside, respectively. The basis has 3 groups of plumose setae inside and these are variable in number. They are 2–3, 3–4 and 1 in specimens examined.

THIRD MAXILLIPED (Mp_3). The exopod has 6 terminal plumose setae: 4 apical and one at each side as in the 2nd maxilliped. The endopod has variable numbers of setae on the sections from the apical to the 5th as follows: 2–4, 3–5, 1, 0–1, 1–2. One to two plumose setae are on the basis inside.

PEREIOPOD 1 (P_1). The propodus is now well differentiated from the carpus and the opposable dactyl can possibly form a holding organ. A couple of setae are near the insertion of the dactyl and a small rudimentary exopod is present on the basis.

P_2 is chelate and much shorter than the others. P_3–P_6 are subequal in size.

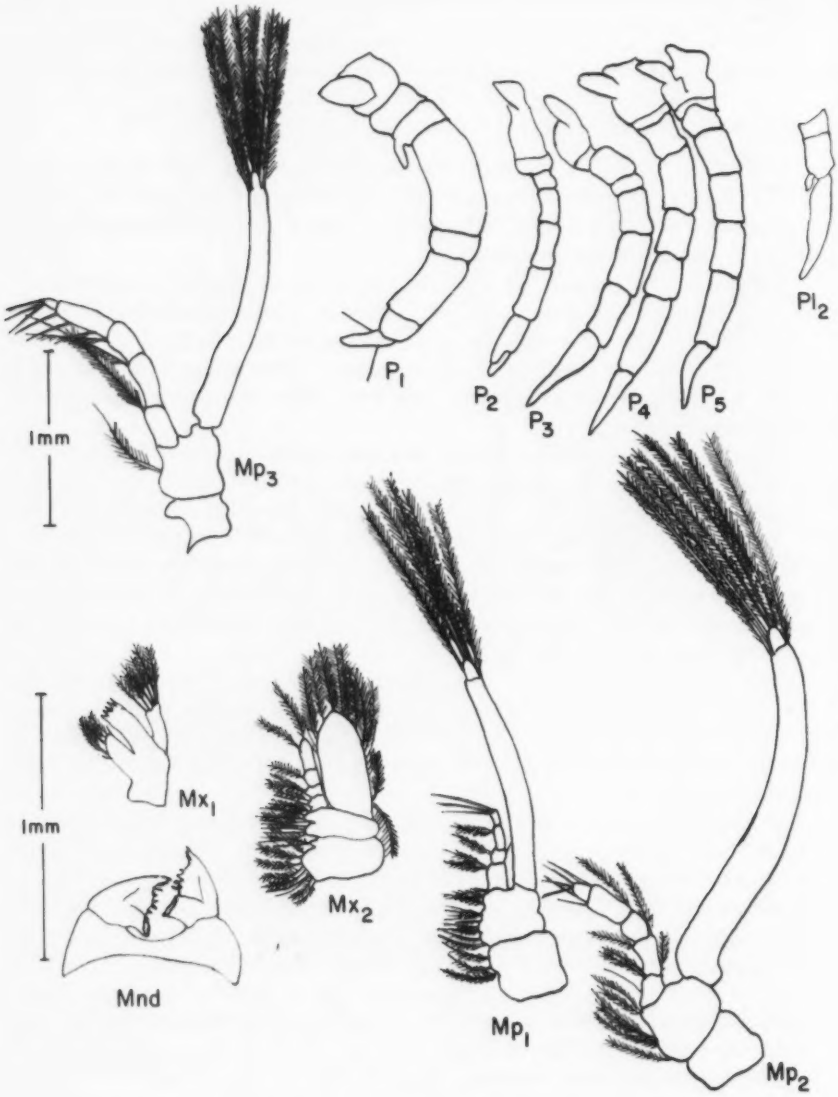

FIG. 4. Stage II larva of *A. dentata*. Lettering for appendages is the same as in Fig. 2.

PLEOPODS (Pl_2). The pleopods of the Stage II zoea have buds of the endopods slightly greater in size than those of the Stage I zoea.

MEGALOPA

Nectocrangon lar Stephensen, 1916 = *Argis dentata*

Stephensen's young stage was 11–12 mm in length and was taken off Greenland in shallow water. An abridgement of his description follows:

"Integument without sculpture; 2 spines mid-dorsally behind the rostrum; spine beneath the eye and at the anterior corner of the carapace; telson has almost parallel sides with 2 pairs of lateral spines near the end, at each side distally of telson are 2 spines of which the inner is much the longer, the centre of the end of the telson projects slightly but is truncate and armed with 2 spines, and in some cases a seta is found between the projection and the spines at the corner." An additional seta is shown on the specimen in Stephensen's figure 3, p. 245 (1916).

Ungava Bay specimens were 10–12 mm in total length and about 2.5 mm in carapace length (Fig. 7). No essential differences from Stephensen's description and figure were found (except numbers of setae on some of the appendages) but a detailed description of the megalopa is as follows (see also Fig. 5 and 6): The carapace has a short pointed rostrum reaching about half the eye. The anterior lateral edge on each side has an ocular, a branchiostegal, and a pterygostomian spine. The carapace edge has a fringe of short fine plumose setae. Dorsal carinae or lateral spines are entirely lacking on the abdomen but a fringe of fine setae is present along the edges of the pleura. The larger terminal spines of the telson are sparingly plumose.

FIRST ANTENNA (Fig. 5, A_1). A very wide expansion is present outside on the proximal section. This expansion has an acuminate point distally and a fringe of about 12 simple setae. Distal to the expansion a cluster of short setae and a few scattered setae are also present on this section outside and at the distal end about 4 short plumose setae. A bunch of about 6 plumose setae are outside also at the distal end of the succeeding section. Inside, the 3 sections bear distally 2 simple, 1, and 1 plumose setae, respectively, from proximal to distal sections. On the lower surface of the middle of the 1st section of the antennule is a strong spine. The two flagella are short, the outer one with 3 sections and bearing apically 3–5 simple setae, and the inner of 4 sections bearing a few plumose setae on the terminal 3 plus 1 plumose and 2 simple setae apically.

SECOND ANTENNA (Fig. 5, A_2). The antennal scale has a large fan of 30–35 long plumose setae inside. The spine of the scale just reaches the tip of the blade. The flagellum is attached obliquely to the second of the 2 sections of the antennal peduncle, and it consists of 1 short and 2 long sections followed by about 12 annulations. The distal section of the antennal peduncle has one strong spine near its outer edge below.

MOUTH APPENDAGES (Fig. 6)

MANDIBLES (Mnd). No palp or incisor process is present. The molar consists of 2 pairs of very sharply pointed cusps almost equal in size but with

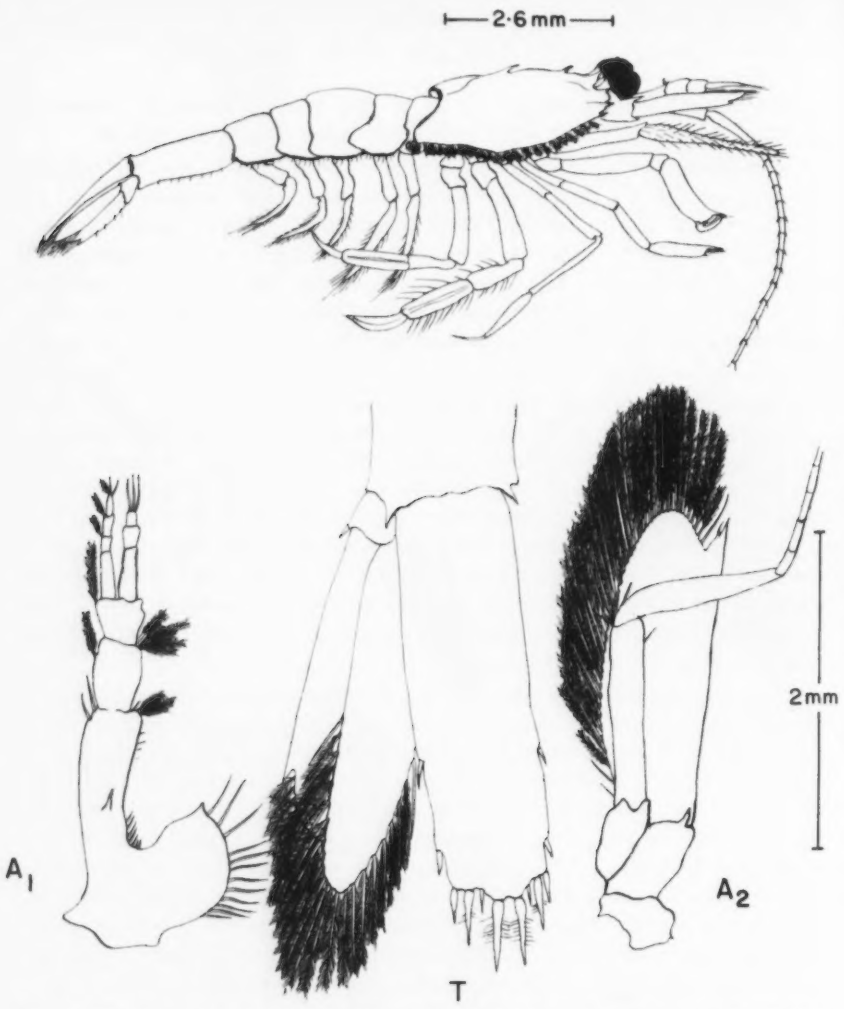

FIG. 5. Megalopa of *A. dentata* from Ungava Bay (2.3–2.6 mm in carapace length; 10–12 mm in total length). Lettering is the same as in Fig. 1.

the inner pair slightly longer. Two or more small accessory cusps per pair are present.

FIRST MAXILLA (Mx_1). The endopod (barely forked) has one simple seta distally. The coxa and basis have a terminal fringe of spines and plumed setae. The coxa has about 4 setae one of which is spinelike, and the basis has about 11 setae, 6 of which are spinelike and slightly recurved and arranged in 2 irregular rows.

SECOND MAXILLA (Mx_2). The exopod has a fan of approximately 35 setae of which the outside lateral setae are mostly non-plumose, but the proximal expansion has about 6 long plumose setae. The endopod is small and has 1–2 simple setae distally and 1–2 short plumose (or simple) setae on its short proximal branch.

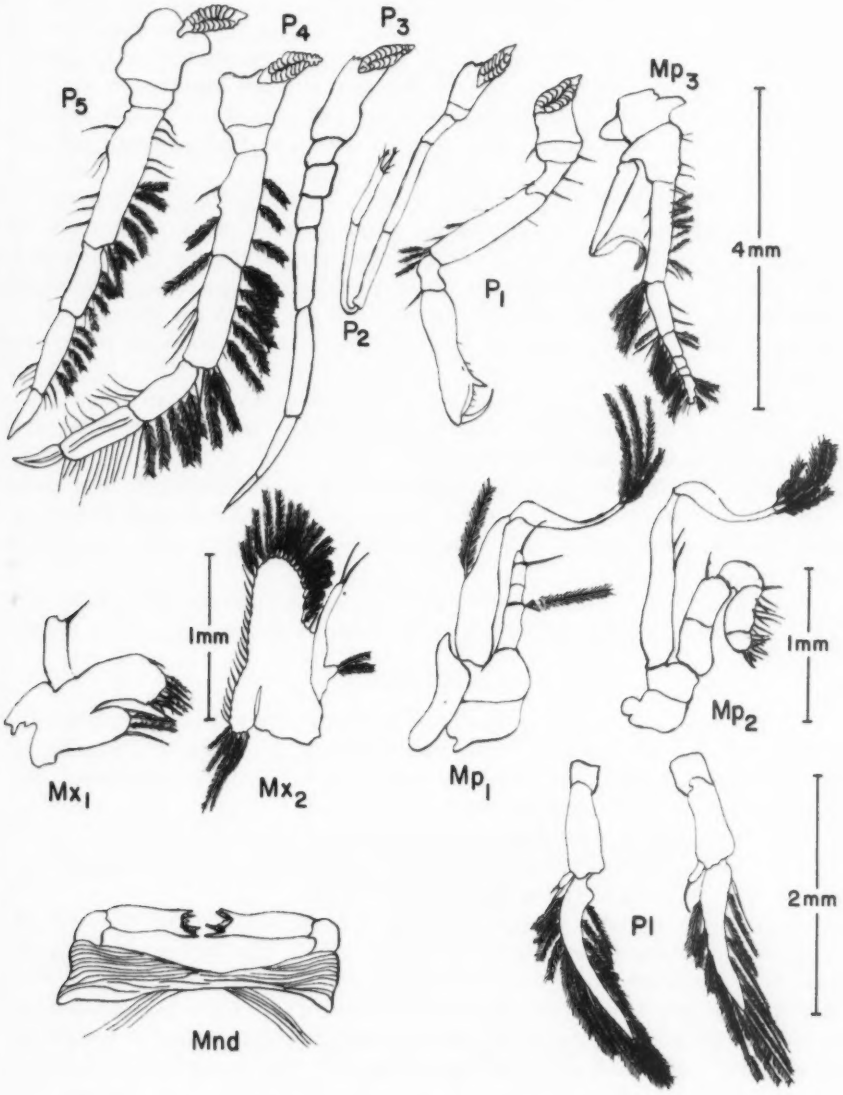

FIG. 6. Megalopa of *A. dentata*. Lettering for appendages is the same as in Fig. 2.

FIRST MAXILLIPED (Mp_1). The exopod is in 2 sections both of which exceed the endopod in length. The proximal section has a long plumose seta outside and the distal section 4 plumose setae distally. The 4-sectioned endopod has few setae—1 plumose seta distally on the 1st or longest section and 1 simple seta on each of the 3rd and terminal sections. An epipod about as long as the coxa and basis combined is attached to the coxa.

SECOND MAXILLIPED (Mp_2). The exopod is in 2 sections, the distal of which bears 6 plumose setae terminally. The 5-sectioned endopod has a permanent flexure at the 3rd section. The 2 terminal sections have strong setae on their inside edges, the distal section about 10 and the other about 8. The 2nd and 3rd sections each have 1 seta distally. The coxa bears a small rounded projection.

THIRD MAXILLIPED (Mp_3). The exopod is in 2 sections and bears terminally on the distal section 5 comparatively small plumose setae (the plumes are not shown in Fig. 6). After the 3rd section the endopod appears to have several divisions which may be annulations. These have many plumose and occasionally simple setae. Outside and distally the proximal section of the endopod has a clump of about 5 long plumose setae. The coxa has a rounded projection outside.

FIRST PEREIOPOD (P_1). This has a well-developed subchelate claw.

SECOND PEREIOPOD (P_2). This is considerably more slender than the others and is simply chelate.

THIRD TO FIFTH PEREIOPODS. The third is a walking leg with a small finely-pointed dactyl. The fourth and fifth have the sections dactyl to ischium flattened in cross-section and many plumose or simple setae along the anterior–posterior edges.

PLEOPODS (Pl). The exopod is well developed, somewhat annulate, and with long plumose seta. There is a short endopod terminating in 1–2 simple setae.

DISCUSSION

IDENTIFICATION OF *A. DENTATA* LARVAE FROM THE PLANKTON. The subchelate 1st pereiopod suggested at once its belonging to the family Crangonidae. A few other diagnostic characteristics making determination definite are (see also Gurney, 1942; Williamson, 1960):

a. A pair of spines on the 5th abdominal segment only,
b. Three denticles on the anterior ventral edge of the carapace,
c. No supraorbital spines.

The only other adult crangonids taken in Ungava Bay were *Sclerocrangon boreas* and *Sabinea septemcarinata* (Squires, 1957). The larvae of the latter have been described already (Sars, 1890; Williamson, 1915; Stephensen, 1916) and they are very distinctive. The larvae of *S. boreas* are not planktonic but are hatched in the adult form with the last 2 legs modified for clinging to the parent (Wollebaek, 1908). It is suggested, therefore, that the crangonid larval Stages I and II taken in Ungava Bay can be none other than *Argis dentata*. The megalopa described here (and by Stephensen, 1916) has many of the adult

characters of *A. dentata* and belongs unquestionably to this species. However, it still retains features found in Stage II at least of the present larvae.

CHARACTERISTIC FEATURE OF THE LARVAE APPEARING IN STAGE I, STAGE II, AND THE MEGALOPA. Apart from the apical dactyl of the 1st pereiopod (a precursor for forming the subchela) as mentioned above, the comparatively small chelate second pereiopod is present in all stages. The outer expansion on the 1st section of the antennule appears in Stage II and is large in the megalopa and adult. The pleopod has a small budding inner projection from the basis even in Stage I and this increases in size in Stage II and in the megalopa. The endopod of the pleopods in this species is reduced even in the adult.

SEASONAL APPEARANCE OF THE LARVAE IN UNGAVA BAY. During the period of investigation the first plankton hauls of the season were made on June 24, and only Stage I of the larvae of *A. dentata* was taken. Stage II appeared in catches of July 3. The latest date at which Stage I was taken was August 2. Stage II was present until August 26. Stages III and IV of *A. dentata* were not taken although plankton hauls continued until August 29. The megalopa of this species

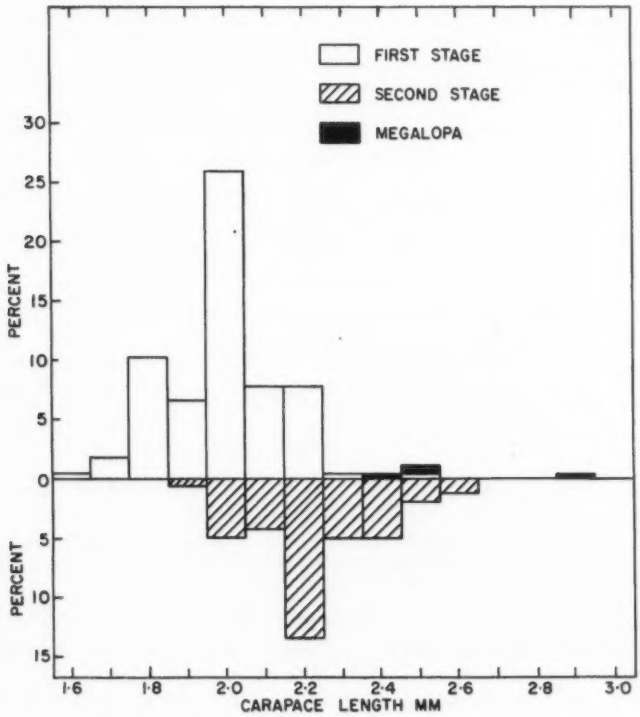

FIG. 7. Lengths of Stages I and II larvae and megalopa of *Argis dentata* taken in Ungava Bay from June 24 to August 20, 1947-50.

was taken from August 11 to 20. Since the morphological differences between Stage II and the megalopa are considerable, and further changes in at least 2 moults would be required to bridge these differences, it is probable that Stages III, IV, or V were present also in late August but were not captured by the plankton nets.

LENGTHS OF LARVAE. Generally there is only a small amount of increase in length between moults into Stages I, II, and the megalopa (Fig. 7), but the megalopa is much bulkier in appearance than Stages I and II larvae. The average and range of lengths were as follows:

Larval stages	Average carapace lengths	Range of carapace lengths
	mm	*mm*
Stage I	2.0	1.6–2.3
Stage II	2.2	1.9–2.6
Megalopa	2.6	2.3–3.0

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Post-Mortem Glycolytic and Associated Changes in the Muscle of Trap- and Trawl-Caught Cod[1]

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ABSTRACT

Glycolytic activity in the muscle of heavily-feeding trap-caught cod (*Gadus morhua*) during the struggle involved in catching, and the subsequent post-mortem changes during holding in ice and at ambient temperatures were investigated. Struggling of the fish in the trap and during boating was sufficient to cause a partial depletion of the muscle energy reserves, as indicated by lactate production and nucleotide dephosphorylation and deamination. One cod from the trap, boated and killed without apparent struggling, had a high initial glycogen content of 550 mg/100 g. During subsequent storage of the brailed fish in air or ice the glycolytic processes, including nucleotide degradation, continued, although during the early post-mortem period the rate of lactate production greatly exceeded that of glycogen depletion. Little change in concentration of the metabolites occurred on freezing and storage at −26°C for 4–6 weeks. In contrast, trawled offshore cod and haddock, regardless of ante-mortem treatment, contained little or no glycogen; almost complete dephosphorylation and deamination of the nucleotide compounds had occurred during the struggle involved in catching. Therefore, at the time of boating, the energy reserves in the trawled fish have been exhausted or nearly so.

INTRODUCTION

The nature of the extensive biochemical and physical changes occurring in muscle post mortem, with particular reference to mammalian muscle, and the rate and extent of these changes as influenced by such ante-mortem conditions as rest and exercise, feeding and starvation, and by post-mortem conditions such as temperature, have been the subject of detailed reviews by Bate-Smith and Bendall (1956) and by Bendall (1960). In recent years, numerous investigators have contributed to the growing elucidation of these processes in commercial fish species. The early work of MacLeod and others (MacLeod and Simpson, 1927; Leim *et al.*, 1927; Macpherson, 1932) revealed initial glycogen levels of 0.5% or more in "rested" muscle (somewhat lower than most values reported for mammalian tissue) and decreased amounts of glycogen with formation of lactate depending on degree of struggling during capture. Since then, many workers have studied one or more aspects of glycogen breakdown, lactate accumulation, and nucleotide dephosphorylation and deamination in relation to method of killing, degree of struggling and exercise prior to death, as well as holding temperature after death (see reviews by Black *et al.*, 1961; Amlacher, 1961; Tomlinson and Geiger, 1962). Influenced by the storage temperature certain of these processes may continue to some extent in frozen fish

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muscle (Larsen, 1944; Jones and Murray, 1961a; Partmann, 1963; Tomlinson *et al.*, 1963).

Some scattered information is available on the post-mortem biochemical changes in the muscle of Atlantic cod (*Gadus morhua*). Jones and Murray (1960) showed that, in rested cod muscle, the major component of the nucleotide fraction was initially adenosine triphosphate (ATP), and that in trawled fish the ATP became almost completely dephosphorylated and deaminated to inosine monophosphate (IMP) during the struggle to exhaustion. These authors (1961b, 1962) subsequently showed that, in rested muscle stored at 0°C, the ATP concentration fell rapidly from 5.34 to 0.2 μmole/g in 12 hr, with a concomitant increase in IMP; in exhausted muscle, IMP present initially in amounts of 4–6 μmole/g was further degraded to inosine and hypoxanthine during the iced storage. Fraser *et al.* (1961) demonstrated that the post-mortem rates of glycolysis and nucleotide dephosphorylation in the muscle of non-feeding, aquarium-held cod were much accelerated at higher temperatures, being completed at 2, 12, and 30 hr at 25, 9, and 0°C, respectively, and that deamination occurred simultaneously with dephosphorylation.

It was the purpose of the present work to extend this study, first, to obtain data on the initial levels of glycogen, lactate, and high-energy phosphate compounds in the heavily feeding cod of the Newfoundland trap fishery, both in rested condition and as commercially handled; secondly, to follow the changes in these metabolites under conditions designed to simulate best and worst possible commercial conditions. For comparison, the initial metabolite concentrations in the muscle of normally feeding fish obtained by prevailing catching methods were examined in samples of trawled cod and haddock subjected to various prefreezing and handling treatments. Since so few studies include data on all the main glycolytic metabolites an attempt was made to study the integrated pattern.

Post-mortem biochemical changes associated with rigor mortis play an important role in determining the quality and characteristics of both the fresh and the frozen product. These changes are dependent upon many factors, including on the one hand, the condition of the fish prior to death, determined by environment, size, sexual maturity, feeding, degree of struggling during catching; and on the other hand, the post-mortem treatment, such as holding temperature, rate of cooling, physical handling, and time elapsed to freezing. These factors assume particular importance in the processing of very fresh fish, as encountered on factory ships or in a shore fishery, where all or part of the catch may be processed prerigor or before rigor has been resolved. A special case in this situation is the annual Newfoundland trap cod fishery.

Special Factors in Relation to the Trap Cod Fishery

The cod (*Gadus morhua*) involved in the trap fishery spawn on the Grand Banks mainly during May, and then commence feeding voraciously on capelin (*Mallotus villosus*). The latter proceed toward the shores of Newfoundland to

spawn from mid June to August (Templeman and Fleming, 1956, 1962). The feeding cod follow the spawning capelin and, where water temperatures are suitable, may be caught in large trap nets set near the coast (for a description of cod traps and trap fishing in general, see Ronayne, 1956). Relatively small cod, 4- to 7-year-old fish, are the principal contributors to the trap fishery (Anon., 1963). The trap fish, therefore, have been feeding heavily on capelin for from 1 to almost 3 months over the trap season. According to Dr W. Templeman (personal communication) the trap cod are fairly well recovered from spawning, having large livers containing about 60% fat. In such heavily feeding fish it might be expected that the muscle glycogen content would be high.

The fish usually inhabit the deep, relatively cold water layers into which the bottom of the trap extends. However, the surface water at the trap may be considerably warmer, 5–10°C, perhaps even up to 15°C in sheltered areas on the south coast of Newfoundland. Thus, the cod may be exposed to warm water for several hours during the raising of the trap net and the brailing of the fish into the boat. The warming of the muscle is assisted by the struggling which takes place during the boating, and in addition, heat would be produced by glycolysis in the muscle. The warming is further enhanced during transfer of the gorged cod from the trap to the plants. This journey in small boats takes several hours and ice is seldom used either prior to or following gutting. Further delays may occur at the filleting and freezing plants, which may be unable to cope efficiently with the glut of fish waiting to be processed. Problems experienced in the plants arising from the excessive drip associated with processing delays and poor handling techniques with these heavily feeding prerigor cod have already been defined (Dyer, 1960; Dyer and Fraser, 1961). Higher temperature and rough handling accelerated markedly the rate and extent of rigor mortis, stiffening, contraction of the muscle, and development of drip. However, an accurate definition of the condition of the raw material, and the effect of various handling conditions post mortem on the biochemical processes associated with rigor mortis, is necessary if the problem of uncertain quality in the frozen product processed from Newfoundland trap cod (Dyer *et al.*, 1962) is to be completely understood and subsequently resolved.

It will be apparent that many of these factors, condition, temperature, struggling, time in the net and in the boat, etc., will vary greatly from area to area and from the beginning to the end of the season. Thus, there is no "standard" trap fish, and in so far as it is influenced by these factors quality may also be expected to vary.

It was planned therefore to sample trap-caught cod as rested as possible, along with those handled with the usual amount of struggling. Some of the latter were left at air temperature in the boat (commercial practice), others were cooled by placing them in crushed ice, while a third lot was cooled as rapidly as possible by immersing in a mixture of ice and water. It was planned to sample these during rigor mortis and after rigor mortis had passed.

EXPERIMENTAL

Physical Methods

Temperature of the interior muscle was measured by inserting the stem of a dial thermometer into the flesh. The pH was measured on a small fresh cut about 1 cm deep in the thick part of the dorsal muscle. The fork length of the fish (tip of snout to fork of tail) was recorded.

Sampling, Extraction, and Biochemical Analyses

In all unfrozen trap cod, a 20–30-g portion of muscle was rapidly excised from the deep anterio-dorsal area close by the first dorsal fin, care being taken to sample all fish in exactly the same position. As soon as excised, the samples were dropped into a polyethylene bag and immersed in an ice–water mixture contained in an insulated chest until extraction could be carried out in the laboratory, up to 4–5 hr later. Preliminary investigation concerning the stability of the metabolites post-mortem had indicated that considerable glycolysis, and nucleotide dephosphorylation and deamination, occurred in muscle samples subjected to slow freezing by placing in polyethylene bags in an ice–salt mixture, and also in portions held, either whole or diced, in cold 3% perchloric acid. In samples of rested cod muscle containing initial glycogen and acid-labile phosphorus levels of about 150 mg/100 g and 10 μmole/g, respectively, glycogen decreased by 20–70% and acid-labile phosphorus by 25–60% of the initial values in 4–5 hr under these conditions. At the same time, lactate increased by about 50%. Similar samples of 5–10 g, frozen by dropping into liquid nitrogen and extracted by blending with ice-cold perchloric acid, showed a 30–40% decrease in acid-labile phosphorus and a 50% increase in lactate, presumably due to thawing of the sample before penetration of the tissue by the acid was achieved during the blending. The minimum change (up to about 20% in 4–5 hr) was found to occur in small muscle samples rapidly cooled and held in ice-water. Since this work was completed, Heslop (1964) found that no dephosphorylation of ATP occurred in samples of insect tissue frozen in liquid nitrogen and powdered with perchloric acid in the presence of excess liquid nitrogen prior to blending at 0°C. However, he found a dephosphorylation to take place if the liquid nitrogen frozen samples were allowed to warm above −3°C prior to refreezing in liquid nitrogen and treatment as above. In agreement with our results, he also found a very slow change in the high-energy phosphate content of unfrozen material held at ice temperature.

For the extraction, a 10-g portion of the muscle sample was rapidly weighed and homogenized (most samples in a Virtis homogenizer) with 60 ml of ice-cold 3% perchloric acid for 3 min, followed by filtration. The residue was reblended with 20–30 ml of 3% perchloric acid. The combined filtrates were adjusted to a volume of 100 ml, and neutralized immediately to pH 7.0–7.5 with 30% KOH to minimize decomposition of labile phosphorus compounds. The extracts of unfrozen trap cod samples made in St. John's were packed in ice and air-

expressed to Halifax, where they were frozen and stored at −26°C until analysis could be completed.

Glycogen was precipitated with ethanol according to Carroll *et al.* (1956), followed by colour development with the phenol-sulphuric acid reagent of Montgomery (1957). Lactic acid was estimated by the method of Barker and Summerson (1941). Ammonia was determined by the micro-diffusion method of Conway (1962), followed by colour development in Nessler's reagent. Total nucleotide (including any small amounts of nucleosides and free bases which might be present) and the relative proportions of adenosine and inosine compounds were calculated from ultraviolet absorption measurements by the method of Szentkirályi (1957). Total phosphorus (TP) in the extracts, inorganic phosphorus (IP) including phosphocreatine phosphorus in the unhydrolyzed extracts, and the increase in IP following hydrolysis in 1*N* HCl at 100°C for 7 min (acid-labile phosphorus, Δ7P) were estimated according to Harris and Popat (1954). The acid-labile phosphorus is derived chiefly from ATP, but labile phosphate esters of non-nucleotide origin may also be present (Bendall, 1960; Burt, 1961).

Trap-Caught Cod

Cod (*Gadus morhua*) were obtained from a commercial trap situated at Cape Bay, about 30 min by boat from St. John's harbour, in the early morning of July 7, 1962. The sea temperature (surface) at the trap was 6°C, and the air temperature was 7°C. Conditions were foggy, and a high sea was running. As the trap, hauled by the fishermen, approached the surface and the mass of fish became visible in about 1 fathom of water, attempts were made with a dip net to obtain several "rested" fish; however, due to the heaving of the boat in the rough sea, only one fish could be obtained without some degree of struggling in the net during transfer. In a calm sea, it would probably have been possible to obtain more "rested" samples, although most cod in the trap were obviously excited and attempting to escape. All other fish used in the experiment were transferred, rather roughly, from the trap to the boat as in commercial practice by brailing, i.e. by scooping with a net or with the hands. These trap cod all had distended abdomens; later examination revealed the stomachs gorged with undigested capelin. The flesh of the fish appeared to be firm, although the distended belly gave an impression of softness in some cases. Several fish held at ambient temperatures were still firm, showing no softness, 8 hr later.

The trap catch was about 3000 lb gutted weight, mainly small to medium-sized fish. It was noticed that a very few small fish went into rigor in the boat prior to landing; most showed no signs of rigor stiffness 30 min to 1 hr after brailing. The commercial part of the catch was gutted at the fishermen's "stage" or small wharf about 1½–2½ hr after brailing, and then taken by boat, still uniced, to the freezing plant where they were forked into "payloaders" and thence to the filleting line. Filleting thus took place about 4–5 hr after brailing, and the fillets went into the freezer about 30 min later.

Cod Sampled at the Trap

To establish the initial level of glycolytic metabolites, the one "rested" live fish together with five brailed live fish were killed by stunning and sampled immediately, within a few seconds of boating (these were the only fish so killed; all others were allowed to die in air). The samples were immediately cooled, as described under "sampling", and extracted 4–5 hr later. The muscle temperature was 6–7°C. These fish were iced for later measurement of length and pH. The interior muscle temperature of two fish after 30 min of icing was 2 and 5°C.

Cod at Ambient Temperature

Twelve fish were allowed to lie at ambient temperature (7–12°C) in the bottom of the boat, as in commercial practice, until landed. The flesh temperature 30 min after brailing was 6°C. The fish were held at the wharf in a metal box, and length, temperature, and pH were measured at 2 hr and at 3 hr after brailing. At 3 hr six fish (muscle temperature 10–12°C) were sampled and the samples cooled in ice–water until extracted 1–2 hr later. At 4 hr these six fish were filleted at a local fish plant, and placed in the freezer about 15 min later. Also at 3 hr the other six fish were moved into warmer air (20°C) for an additional 2 hr, after which they were measured (muscle temperature 18–19°C), sampled, and the samples extracted immediately, a total of 5 hr after brailing. These fish were filleted at about $5\frac{1}{4}$ hr, and frozen about 15 min later. It was not possible to delay sampling until the fish had entered maximum rigor, as planned.

Iced Cod

Twelve fish were well iced in metal boxes immediately after brailing. Physical measurements and prerigor muscle samples from six fish (flesh temperature 0.5–2°C) were taken at $2\frac{1}{2}$ hr; the muscle samples were cooled and extracted $1\frac{1}{2}-2\frac{1}{2}$ hr later. These fish were filleted and frozen at 4 hr. At 5 hr the remaining iced fish were gutted, washed, and well iced in boxes at the fish plant. After 48 hr these were sampled, filleted, and frozen. At this time the flesh had become soft and sticky, with off-odours present; most of the fish were out of rigor.

Cod in Ice–Water

Another lot of 13 fish was placed after brailing into a seawater–ice mixture at approximately 0°C. The purpose was to cool the fish as rapidly as possible. It was noted that some of these fish struggled occasionally during the trip in to the wharf. Six fish were measured and sampled at about $2\frac{1}{4}$ hr after brailing; the muscle samples were cooled and extracted $1\frac{3}{4}-2\frac{3}{4}$ hr later. These fish were filleted and frozen at 5 hr. The remaining fish were gutted at about $5\frac{1}{2}-6$ hr after brailing and well iced at the fish plant. After 48 hr most were out of rigor, and the flesh had become very soft, sticky and mushy, with off-odours present. They were then sampled, filleted, and frozen.

Frozen Trap Cod Fillets

Following transportation to a local fish plant, the fish were filleted, the fillets packaged in waxed 1-lb cartons, overwrapped with waxed paper, machine sealed, and frozen in horizontal plate freezers as in commercial practice. The packages went into the freezer about 15 min after packing and remained in the freezer for 70–90 min. The frozen fillets were transported by air to Halifax in insulated cartons containing dry ice (Dyer *et al.*, 1964). Temperature on arrival ranged from −20 to −35°C. The samples were stored at −26°C for 4–6 weeks prior to analysis. For the analyses, a cross-section of the frozen 1-lb fillet block was diced coarsely with a knife and extracted while still hard-frozen. Each of four separate 1-lb fillet blocks, each containing two to three individual fillets, was analyzed.

Trawled Fish

The trawled fish were obtained during two cruises of the Fisheries Research Board research vessel *A. T. Cameron*.

On a trip undertaken in March 1960, samples of muscle weighing approximately 100 g were cut from the anterio-dorsal area of prerigor and in-rigor otter-trawled cod and haddock, enclosed in polyethylene bags, and immediately frozen by immersion in brine at −12°C. The samples were held at this temperature for 3–4 days on board the vessel. On landing, the frozen samples were stored at −26°C for approximately 1 month before extraction.

In March 1963, cod taken in 20-min sets were subjected to three different treatments before sampling: (A) live fish as rested as possible, killed immediately after the trawl was emptied, (B) similar fish allowed to recover by being held alive overnight in seawater, and (C) exhausted fish allowed to struggle to death on deck. Several fish subjected to each of these three treatments were sampled and frozen in brine as above. The frozen samples were maintained at about −12°C for 2 days during transportation to the laboratory, where they were placed in storage at −26°C for 1 week. Representative samples of muscle from three fish in each group were diced with a knife while still hard-frozen, well mixed, and 20-g portions weighed for extraction.

RESULTS AND DISCUSSION

Lengths and other physical measurements of the trap cod examined are given in Table I. Levels of glycolytic metabolites in the anterio-dorsal muscle of trap cod subjected to various post-mortem handling conditions prior to freezing are presented in Table II. Attempts were made to correlate length of individual fish with concentration of the various metabolites in the muscle, but no significant relationship was found. Thus, average values of five to seven fish are reported for each lot, with the exception of the "rested" fish where only one sample was available.

TABLE I. Physical measurements of "rested" and brailed trap cod, and of brailed trap cod held in air, in ice, and in ice–water.

	Rested[a] at trap		Brailed[a] at trap		Ambient			Ice		Ice–water	
					7–12°		3 hr at 7–12° + 2 hr at 20°				2½ hr ice-water + 48 hr ice
Hours post mortem	0	2(ice)	0	2(ice)	2	3	5	2½	50	2½	50
Length: Mean (*cm*) Range *n* SE	–	48 – 1	–	61 41–79 5 ±7	52 43–61 6 ±3	–	47 39–56 6 ±3	56 51–64 7 ±2	47 41–53 5 ±2	52 46–58 6 ±2	42 33–53 7 ±3
Muscle temperature (°*C*)	6°	1°	6–7°	0–1°	7–8°	10–12°	18–19°	0.5–2°	0–1.5°	0°	0–2°
Muscle pH	–	6.7	–	6.7–7.2	6.8–7.2	6.6–6.8	6.4–6.7	6.8–7.2	6.2–6.5	6.8–7.3	6.1–6.4
Stage of rigor	–	pre	–	pre	pre	pre	sl. rigor	pre	post	pre	post
Condition of flesh	firm	firm	firm	firm	firm	firm	slimy, firm	firm	soft, mushy	firm	soft, mushy

[a]Killed immediately by stunning; all others allowed to die.

TABLE II. Glycogen, lactate, ammonia, nucleotides, and P compounds in the muscle of "rested" and brailed trap cod, and in brailed trap cod held in air, in ice, and in ice–water.

		Rested at trap	Brailed at trap	Ambient		Ice		Ice–water	
				7–12°	3 hr at 7–12° + 2 hr at 20°			2½ hr ice–water + 48 hr ice	
Hours post mortem		0	0	3	5	2½	50	2½	50
No. of fish analyzed		1	5	6	6	7	5	6	7
Glycogen mg/100 g	Mean	554	203	197	121	223	50	149	44
	SE		±62	±46	±39	±42	±13	±32	±10
	Range		81–384	83–348	31–251	44–347	13–73	64–257	15–78
Lactate mg/100 g	Mean	166	163	313	359	294	398	245	394
	SE		±14	±31	±26	±22	±30	±26	±40
	Range		130–194	196–380	250–450	236–373	300–470	148–315	180–489
IP μmole/g	Mean	36.5	31.3	40.8	42.2	42.3	46.4	38.1	46.1
	SE		±3.8	±2.7	±1.0	±0.6	±0.6	±5.0	±1.7
	Range		16.8–39.5	28.0–45.4	38.1–44.9	37.0–45.9	44.1–50.8	17.5–48.8	37.9–50.3
ΔTP μmole/g	Mean	12.0	6.42	5.02	3.83	5.66	2.12	6.59	2.69
	SE		±0.86	±0.57	±0.65	±0.67	±0.62	±1.00	±0.59
	Range		3.42–8.45	2.71–6.38	2.19–6.38	3.13–8.16	0–3.87	3.19–9.26	1.39–5.61
Ammonia μmole/g	Mean	5.50	5.49	6.83	6.80	6.43	7.89	6.68	8.10
	SE		±0.41	±0.58	±0.37	±0.29	±0.21	±0.41	±0.39
	Range		4.00–6.22	4.79–9.07	5.21–7.85	5.43–7.65	7.57–8.29	5.93–8.22	6.71–9.30
Total nucleotide μmole/g	Mean	8.07	6.44	6.92	7.23	7.58	7.14	7.69	7.43
	SE		±0.84	±0.86	±0.21	±0.15	±0.85	±0.95	±0.66
	Range		3.27–8.07	2.73–8.35	6.91–8.15	6.95–8.35	3.88–8.81	3.01–9.29	4.01–9.46
Adenine nucleotide Percentage of total	Mean	71.6	39.0	36.9	28.6	37.2	6.8	33.0	9.8
	SE		±3.7	±4.3	±3.3	±2.2	±2.6	±3.6	±1.5
	Range		24.5–44.6	28.8–57.7	14.4–38.8	28.6–45.2	0–14.4	19.3–42.2	4.2–15.6

Trap Cod: "Rested"

The one rested fish examined (Table II) contained a large amount of muscle glycogen (554 mg/100 g), comparable with the few values available in the early literature for unexercised, feeding fish of various species. However, the initial accumulation of a significant amount (166 mg/100 g) of lactate suggests that some glycolytic activity associated with struggling had occurred prior to death, since the lactate level in well-fed cod *completely* at rest prior to death is practically zero (unpublished results). On the other hand, the Δ7P content remained at a high level of 12 μmole/g, equivalent to 6.0 μmole ATP/g. This Δ7P value is similar to that found in rested cod muscle by Fraser *et al.* (1961) and is also comparable to the ATP level of 5.2 μmole/g chromatographically isolated from resting cod muscle by Jones and Murray (1960). It seems likely that most fish obtainable from the trap will be at least partially exercised, since some struggling is unavoidably induced by the massing together of large numbers of fish during the hauling of the trap, especially as it approaches the surface. If the lactate produced in the muscle is taken as roughly proportional to loss of muscle glycogen, then an initial glycogen content of 700 mg/100 g or greater is indicated for the sample of rested trap cod. This level in cod muscle is greater than any so far recorded, although not unexpected in such heavily feeding fish. For instance, large increases in the muscle glycogen of rainbow trout as a result of heavy feeding have been reported by Hochachka and Sinclair (1962).

Trap Cod: Brailed

In the fish hauled and removed from the trap net under the conditions of commercial practice, i.e. by brailing, and sampled immediately, the glycogen content averaged 200 mg/100 g. In two of the five fish analyzed the glycogen remained at a high level, 317 and 384 mg/100 g. The accompanying average lactate level of 163 mg/100 g indicates partial glycolysis, as found in the rested sample (see previous section). The glycolysis, a result of both the struggling in the trap and the ensuing handling during boating, was accompanied by partial nucleotide dephosphorylation; the Δ7P fraction was reduced in the brailed samples to a level half that of rested cod muscle (see previous section). The adenine nucleotide content was 39% of the total nucleotide, indicating partial deamination to inosine nucleotide. In contrast, adenine nucleotide in the single rested sample amounted to 72% of the total. The ammonia level, averaging 5.5 μmole/g in the brailed samples, also indicates partial deamination, since initial levels of 2–4 μmole/g have been reported in unexercised cod and other species of fish (Amano *et al.*, 1953; Suyama *et al.*, 1960; Fraser *et al.*, 1961). The neutralized perchloric acid extracts of muscle from the fish sampled immediately after brailing (and of all subsequent samples) had ultraviolet absorption maxima in the inosine nucleotide range, 248–250 mμ, indicating deamination to inosine monophosphate (IMP). However, a maximum of 257 mμ, characteristic of adenosine compounds, was observed in the extract from the one rested fish with high initial contents of glycogen and Δ7P.

The extent of glycolytic change in the muscle during muscular activity has not been well defined in fish, although during complete exhaustion or struggle to death almost complete conversion of glycogen to lactate occurs, with depletion of ATP and its deamination to IMP (Amano *et al.*, 1953; Tomlinson *et al.*, 1961; Black *et al.*, 1960, 1962). In mildly exercised rainbow trout, Black *et al.* (1960, 1962) and Tomlinson *et al.* (1961) found some glycogen decrease and a slight increase in lactate, but the latter found little dephosphorylation; all results, however, were quite variable. Amano *et al.* (1953) also found more glycolytic activity with slight ammonia formation in beheaded frigate mackerel as compared with rested fish killed by severing the motor nerve. The present results indicate that struggling at the trap is sufficient to result in only a partial depletion of the muscle energy reserves.

Trap Cod: Post-Mortem Glycolytic Pattern

During storage of the brailed fish for 2–3 hr in either air, ice, or ice–water, little change occurred in glycogen content (Fig. 1). The amount of lactate, however, almost doubled, from 160 to an average of 290 mg/100 g. It is reasonable to assume that this appreciable lactate production without concomitant loss of glycogen occurred because during the ante-mortem struggle (as previously discussed) the rate of glycogen breakdown was more rapid than the formation of lactate, the end product of the glycolytic cycle, resulting in an accumulation of glycolytic intermediates; then, during the early post-mortem period, the rate

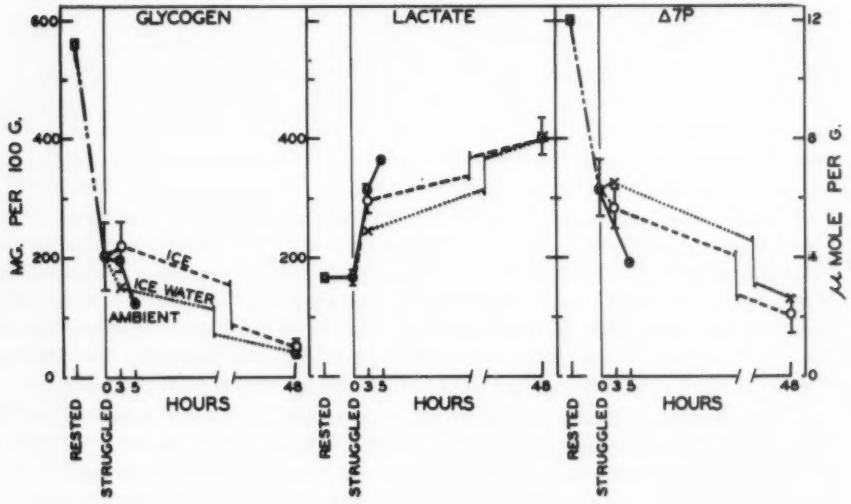

Fig. 1. Glycolytic metabolites in muscle of rested and brailed trap cod, and in brailed trap cod held at ambient temperatures, in ice, and in ice–water. Standard error of the mean is shown for the brailed and the iced samples.

of lactate formation from the intermediates greatly exceeded that of further glycogen depletion. The amounts of glycolytic intermediates necessary for the delayed lactate formation are consistent with the levels of sugar phosphate compounds present at death in cod muscle (Burt, 1961; Burt and Jones, 1961), although little data are available on the changes in concentration of these compounds in the early post-mortem period, or the effect of degree of struggle thereon. Little decrease occurred in Δ7P or in the proportion of adenine nucleotide, although a small increase in ammonia from levels of 5.5 to about 6.6 μmole/g indicated that deamination was continuing during this period. At this time, muscle pH ranged from 6.7 to 7.3 regardless of holding temperature, with most values lying between 6.8 and 7.0 (Table I).

On longer storage, differences in the rate of glycolysis appeared to develop between those samples stored at ambient temperature and above, and those held at ice temperature. More frequent sampling, not possible at the time this experiment was undertaken, would be required to establish the effectiveness of rapid cooling by ice or ice–water in retarding the glycolytic processes. The temperature of the fish held in ice and ice–water was between 2 and 5°C after 30 min, and had reached levels of 0 and 1°C (one reading of 2°C) at 2 hr. At the ambient temperatures, where muscle temperature was 7–8° for the first 2 hr followed by a gradual rise to 18–19° after 5 hr, there was a rapid drop in glycogen with concomitant increase in lactate, and a similarly rapid decrease in Δ7P (Fig. 1). The pH of the muscle at this time had fallen slightly to 6.4–6.7. Onset of rigor was just beginning in these fish, with evidence of slight stiffness in some.

After 50 hr at ice temperature, resolution of rigor appeared to be complete; the muscle pH has decreased to 6.1–6.4, and the post-mortem glycolytic changes were practically completed. Only 44–50 mg glycogen/100 g muscle remained, and lactate had increased to about 400 mg/100 g. The proportion of adenine nucleotide was reduced to less than 10% of the total nucleotide, and along with an increase in ammonia to maximum levels, indicated that dephosphorylation and deamination were complete or nearly so, although at this time about 2 μmole Δ7P/g of muscle remained. According to Burt (1961), the acid-labile phosphorus compounds glucose 1-phosphate and fructose 1,6-diphosphate, which contribute about 2 μmole Δ7P/g initially in rested cod, decreased rapidly to the equivalent of 0.1 μmole Δ7P/g after 2 days in ice. Tomlinson and Geiger (1963) found approximately 0.46 μmole ADP/g muscle bound to actin in lingcod. As well as ATP, therefore, the actin-bound ADP and possibly other labile phosphate esters may be present in the Δ7P fraction of the trap cod muscle.

The results indicate that trap cod after brailing into the boat will still retain a considerable part of the muscle energy stores of glycogen and high-energy phosphate compounds, which may vary with the degree of struggling of the individual fish. As normally landed without ice at the plants, their initially high glycogen content will be much reduced and their supply of high-energy phosphate compounds almost exhausted. This is a result of glycolytic activity accelerated by the handling at the trap, the subsequent holding without ice at

elevated summer temperatures, and possible delays during gutting and during processing at the plants. Many trap cod, therefore, will have already entered rigor at the time of freezing, or will be approaching it. Thus, the conditions of handling coupled with high holding temperatures to which the heavily-feeding trap cod are subjected in commercial practice, and the resultant acceleration of post-mortem biochemical and enzymatic changes, undoubtedly contribute to the poorer initial quality and shortened shelf life of the frozen material (Dyer *et al.*, 1962). In the present experiment, the samples of trap cod held 2 days on ice into the post-rigor period had abnormally soft and mushy flesh, and strong spoilage odours. In contrast, offshore cod iced for the same period are usually in prime condition, showing no detectable deterioration.

Trap Cod: Frozen Fillets

During frozen storage for 2 months at −26°C no consistent change in glycogen, Δ7P, or ammonia occurred, regardless of prefreezing treatment (Table III). A further increase in lactate, averaging 10%, took place in all samples. Larsen (1944) found no significant change in lactate in 2 months storage at −20°C of minced cod muscle rapidly frozen in dry ice. Although an increase may have occurred in certain samples in the delay between sampling for analysis and freezing, most of the 10% increase in lactate found in the trap cod samples probably occurred during the freezing. Results of Tomlinson *et al.* (1963) indicate that in samples of lingcod muscle frozen at the temperature of storage (−10, −20, and −30°C), 50% or more of the total increase in lactate over a period of 30 days storage occurred on freezing (analyzed after 1 day). Although at higher temperatures rapid ATP breakdown occurs, the lack of change in Δ7P and ammonia in the trap cod samples stored at −26°C is in agreement with the results of Partmann (1963), who reported very little ATP breakdown in rainbow trout and carp muscle during 6 months storage at −24°C.

Trawled Fish

The results of examination of cod and haddock caught by otter trawl are presented in Table IV. With one exception these trawled fish contained little or no glycogen, in agreement with earlier observations of MacLeod and Simpson (1927), Leim *et al.* (1927), and Macpherson (1932). No differences were observed in the levels of metabolites in these fish, whether prerigor or in-rigor when frozen (1960 samples), or whether samples (1963 catch) were taken immediately on emptying the trawl (A), allowed to struggle to death in air (C), or held live for 1 day (B). In addition, the levels of metabolites in haddock were not significantly different from those in cod muscle. The high levels of lactate present in the 1960 catch were most likely due to glycolysis during the struggle involved in catching and killing. Lactate levels in the 1963 catch were lower, indicating possibly a lower glycogen reserve in the rested living animal due either to starvation or perhaps to depletion of the reserve during the spawning cycle. Small remaining amounts of Δ7P and adenine nucleotide in the majority of the

TABLE III. Effect of storage for 4–6 weeks at −26°C on glycolytic metabolites in frozen trap cod fillets processed from the fish subjected to prefreezing treatments as in Table I.

		Ambient		Ice		Ice–water	
		7–12°	3 hr at 7–12° + 2 hr at 20°			2¼ hr ice–water + 48 hr ice	
Hours post mortem prior to freezing		3	5	2¼	50	2¼	50
Glycogen mg/100 g	Mean[a]	128	91	222	62	114	99
	SE	±44	±29	±71	±26	±27	±24
	Range	38–223	33–168	56–404	21–137	54–183	47–163
Lactate mg/100 g	Mean	395	419	357	434	329	435
	SE	±36	±15	±32	±11	±9	±19
	Range	291–453	387–454	276–423	407–453	308–349	411–491
IP µmole/g	Mean	41.7	41.7	41.6	44.0	42.2	42.8
	SE	±1.3	±1.0	±1.8	±1.3	±1.4	±2.1
	Range	37.7–44.2	40.6–44.8	39.7–42.7	41.1–47.3	39.5–47.5	39.9–45.5
Δ7P µmole/g	Mean	3.89	4.50	5.38	2.97	5.40	1.63
	SE	±0.41	±0.94	±1.03	±0.72	±0.91	±0.61
	Range	2.87–4.68	1.87–5.94	3.22–7.81	1.45–4.90	3.84–8.03	0.26–3.10
Ammonia µmole/g	Mean	7.06	6.77	6.52	7.22	7.21	6.91
	SE	±0.36	±0.18	±0.27	±0.04	±0.08	±0.27
	Range	6.29–7.93	6.35–7.21	5.93–7.21	7.15–7.28	7.07–7.42	6.57–7.71
Total nucleotide µmole/g	Mean	7.42	7.46	7.30	8.22	7.88	7.38
	SE	±0.21	±0.21	∓0.17	±0.40	±0.19	±0.19
	Range	7.04–7.98	6.95–7.98	6.88–7.67	7.61–9.37	7.54–8.44	6.95–7.78
Adenine nucleotide Percentage of total	Mean	28.2	20.7	28.2	10.4	27.2	11.9
	SE	±1.8	±2.4	±1.0	±1.2	±1.9	±1.0
	Range	25.6–33.6	14.2–25.6	25.8–30.7	7.2–12.6	22.4–31.8	9.5–14.4

[a]Determinations were made on each of four separate 1-lb packages of frozen fillets.

TABLE IV. Glycogen, lactate, ammonia, nucleotides, and P compounds in trawled, frozen cod and haddock muscle.

	Glycogen	Lactate	TP	IP	Δ7P	NH_3	Total nucleotide	Percentage adenine nucleotide
	mg/100 g				*μmole/g*			
1960 Cod: Prerigor								
Fish No. 1	14	215	67.1	55.2	8.19	7.79	7.76	20.0
Fish No. 2	2	160	63.3	58.1	0.97	7.07	7.52	17.4
Fish No. 3	13	261	58.2	52.1	0.00	7.21	9.12	21.9
Cod: In-rigor								
Fish No. 1	175	402	64.2	44.6	8.45	8.36	8.18	22.8
Fish No. 2	5	169	60.5	57.4	0.19	7.29	8.32	16.1
Fish No. 3	3	142	55.8	51.5	0.00	6.07	7.84	12.9
Haddock: Prerigor								
Fish No. 1	–	305	63.4	56.0	0.48	6.88	7.84	20.0
Fish No. 2	7	263	60.9	57.2	2.26	6.59	7.49	21.1
Haddock: In-rigor								
Fish No. 1	–	291	95.2[c]	85.1[c]	2.16	6.40	10.1	15.8
Fish No. 2	5	307	66.5	53.5	11.6	6.93	8.18	16.0
1963 Cod: A[a, b]	22	121	54.8	49.7	0.65	7.78	7.86	7.6
B	22	97	53.5	47.7	0.00	6.94	7.66	11.0
C	18	116	52.3	42.6	0.00	7.64	8.08	7.1

[a]A: Sampled immediately on emptying the trawl.
B: Held alive 18 hr in seawater.
C: Allowed to struggle to death in air.

[b]A mixed sample from three separate fish was used for the analyses.

[c]High values due to presence of some skin in this sample.

trawled fish, and high levels of ammonia, indicated advanced dephosphorylation and deamination of ATP during the struggling on catching. (However, three samples showed inexplicably high Δ7P values, 8–11 μmole/g, even though the ammonia level and proportion of adenine nucleotide indicated almost complete deamination.) Conversion of ATP to IMP during the struggle to exhaustion in trawled codling, with levels of 0.27 μmole ATP/g remaining, has been demonstrated in chromatographic studies by Jones and Murray (1961b, 1962), and by similar studies of trawled haddock, lemon sole, and plaice by Kassemsarn *et al.* (1963). Bramstedt (1962) reported that in exhausted trawled cod there remained only one-sixth of the initial level of 16 μmole Δ7P/g found in cod caught and killed under the best possible conditions ("unter schonendsten Fang-und Tötungs-Methoden"), which, however, were undefined. In a sampling of 78 trawled cod, Partmann and Gutschmidt (1963) reported a median Δ7P value of 3 μmole/g with a range of 0–10 μmole/g. However, the role played by the freezing process in accelerating glycolytic changes, especially at temperatures such as those used for the trawled fish in the present study (–12°C), may be significant. During slow freezing glycolysis and ATP splitting occur at an appreciable rate down to at least –10°C, with maximum rates at about –3°C (see review by Tomlinson *et al.*, 1963).

The absence of glycogen in trawled fish is in contrast to the average level of 200 mg/100 g found in brailed trap cod. The trawled fish (1960 samples) also had significantly lower average adenine nucleotide content than the brailed samples, 18 vs. 39%, respectively, and higher ammonia levels, 7.1 vs. 5.5 μmole/g. Lactate levels were also higher in the trawled fish, 250 vs. 160 mg/100 g. Most Δ7P values for the trawled fish were nearly zero, in comparison with an average level of 6.4 μmole/g in the brailed trap cod.

These findings indicate that the trawled fish have become exhausted or nearly so due to struggling and handling during the dragging and emptying of the trawl net, and that little further glycolytic change occurs even though the fish are allowed to struggle to death on deck (Sample C, Table IV). However, as has been pointed out above, there is some uncertainty as to the extent of the glycolytic change attributable to the freezing conditions used. In contrast,the glycogen and high-energy reserves of the brailed trap cod are only partially depleted during the hauling of the trap net and the brailing into the boat. Thus, in these fish sufficient reserves are present to allow post-mortem glycolytic reactions to proceed. Post-mortem temperatures and handling conditions may modify these reactions and affect subsequent quality of either the iced or the frozen product (Dyer and Fraser, 1961).

It is reported (Jones and Murray, 1961b) that the onset of rigor in exhausted trawled cod occurs some 3 hr earlier than in rested fish. Since the level of ATP in these exhausted fish at death has already reached the point where onset of rigor usually occurs, it is evident that our knowledge of the factors controlling the onset of rigor mortis is far from complete and that ATP level may not be the only vector involved. A more definite correlation of ATP level at death with time to onset of rigor in trawled fish is required. For instance, some fish do appear to go into rigor almost immediately after death, whereas the onset may be delayed for considerable periods in others. Likewise, some high-energy phosphate remained in some of our samples of trawled cod and haddock, but more data are necessary to establish how time of onset is related to the Δ7P fraction.

In practice, therefore, more careful handling of the fish during catching may result in some delay of the onset of rigor, and allow more time for filleting and processing in the case of freezing at sea or handling of trap cod. On the other hand, the presence of significant amounts of glycogen and high-energy phosphate compounds in such carefully handled fish might mean that thaw rigor would become a serious problem when the fish were thawed. However, Banks (1962) considers that thaw rigor often does not present a serious problem in sea-frozen fish.

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Reproduction of King Crabs, *Paralithodes camtschatica* (Tilesius)[1]

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ABSTRACT

Sublegal, post-molt, male king crab graspers are capable of reproducing and may suffice as brood stock in the male-only king crab fishery. Observations in tanks revealed that the younger males bred successfully with an average of five females.

INTRODUCTION

Included in this report are: a review of Japanese, Russian, and American literature on king crab reproduction; observations of breeding in the natural environment; and results of studies of the reproductive potential of captured breeders placed in aquaria.

The purpose of this study was to determine if recently molted, sublegal, male crabs captured while grasping mature females in the natural environment were able to breed and, if so, with how many females could they mate. The male-only commercial fishery maintains a size limit of 178 mm carapace width (including spines). This size limit revealed the importance of learning if crabs too small for harvest were capable of perpetuating stocks reduced of legal males.

The literature review is included for ready reference. Available information on king crab reproduction is scattered through various publications, and appears in three different languages.

The investigation did not attempt to compare or survey spawning grounds, but rather conducted observations only on coastal reefs where breeding was first encountered. These data are limited because the extent that the study area represents breeding areas in general is unknown. Ocean areas and substrates such as mud and sand may also be utilized.

LITERATURE REVIEW

Time of Breeding

Most authors agree that king crabs migrate to shallow water to spawn during April and May (Marukawa, 1933; Rumyantsev, 1945; Vinogradov, 1945; Wallace *et al.*, 1949; Powell, 1958; Bright *et al.*, 1960; Kurata, MS, 1961; Powell, 1964).

Kurata is believed to be the author of the anonymous section devoted to king crab research by Japan in the 1961 International North Pacific Fisheries Commission Annual Report. Kurata's preliminary translation (I.N.P.F.C.

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document 481) presents the same data with improved analysis; therefore, the present report relied upon document 481 rather than the anonymous section in the annual report.

Wallace *et al.* (1949) state; "as the molting season approaches, the males first and then the females migrate into shallow depths, where in certain areas they can be found congregated in large numbers." According to Kurata (MS, 1961), breeding crabs form the greatest concentrations in coastal waters rather than in ocean shallows, and young adult females breed in April followed a month later by the older females.

Powell (1964) states that king crabs begin their migration to shallow coastal areas during December and continue through April. Young adults precede old adults; males precede females. The majority of sexually mature males molt from February through April while enroute to the breeding grounds (a small percentage molt in December and January), but the females molt on the breeding grounds from late March to late May usually while being grasped by a male. Anexuviant males were congregated on the spawning grounds among the female breeders (Powell and Gray, MS, 1964). Miyahara and Shippen (MS, 1963) also found "... large non-molting male crabs congregated in relatively shallow water for the purpose of mating. . ."

Size at Sexual Maturity

There is no known method at present to determine sexual maturity of males except by microscopic examination of the reproductive tract, though some authors indicate the contrary. Grasping males captured by divers are probably mature crabs since they are always large. Gravid females are obviously mature. Kurata (MS, 1961) states that, "the right chela, particularly the propodus, of adult males and the merus of females shows a special type of growth in relation to sexual maturity," but he does not document his findings. His measurement data were analyzed by using the relative growth equation $Y=bx^{\alpha}$ and plotted on log/log paper, but the data were not subjected to statistical analysis, and no tables were presented to show the size range and frequency of juvenile crabs from which he made distinctions on relative growth. At present these data are too unrefined to be of practical use. A statistically sound method to determine sexual maturity using proportional growth of body parts would be useful and would not require killing the crabs.

Length of carapace at sexual maturity varies among areas. Carapace width measurements for both sexes from the coasts of Kamchatka and the sea of Nemuro are provided by Marukawa (1933), but have been converted to standard length by Wallace *et al.* (1949) for easy comparison. Wallace *et al.* (1949) state that lengths of crabs off the coasts of Kamchatka and in the sea of Nemuro range from 78 to 82 mm and 92 to 96 mm, respectively. In the Bering Sea they range from 86 to 102 mm, and in the Pacific Ocean from 93 to 112 mm. More recent data collected by Powell (MS, 1960) in the vicinity of Kodiak Island is compatible. The smallest ovigerous female among 3468

individuals examined in Chiniak Bay during April and May 1958 was 95 mm in length. Only 4 females larger than 118 mm were without eggs. Although the minimum size at sexual maturity may be as low as 83 mm in rare cases, as found by Nickerson in December 1963, the authors consider a practical average for the biological minimum length for both sexes to be approximately 100 mm. These data assume that males become sexually mature at the same size as females. Wallace *et al.* (1949) microscopically examined a few male reproductive tracts which tended to support this hypothesis.

The largest male king crab captured to date is from the Kodiak area and has a carapace length of 227 mm and a width inside the spines of 283 mm. The dimensions of the largest female, also from Kodiak Island, are 195 mm in length and 213 mm in width. The crabs were captured in Jap Bay and on the Portlock Banks, respectively.

Precopulatory Embrace

The precopulatory embrace is an intrinsic behavior characteristic of adult king crabs and numerous other crabs including, specifically, the local dungeness, *Cancer magister*, and tanners, *Chionoecetes bairdi;* however, the manner, position, and duration of grasping probably differs among the above species.

Adult females preparing to breed and prior to ecdysis are believed to attract male breeding partners by emitting a chemical stimulant such as that described among lobsters (Hughes and Matthiessen, 1962) and for shrimp (Burkenroad, 1947).

The male king crab grasps the female by the meropodite of both chelipeds while in a face to face position. Exceptions to this basic pattern have been noted by Bright *et al.* (1960) and the authors of this manuscript, but never in the natural environment.

The precopulatory embrace seems to have three benefits to the species; keeping breeding adults together until subsequent mating has occurred; affording a protective mate to the female before and during molting; and aiding the female to exuviate.

The precopulatory embrace lasts throughout the pre-molt and molt stages; the pre-molt being of longer duration. Marukawa (1933) states that grasping may last for 3–7 days before the female exuviates. The longest duration of grasping observed in the present study was 3 days; however the length of grasping before capture is not known. Seven days would appear to be a maximum figure. The size of grasping partners appears to be unrelated since male king crabs mate with females much smaller or larger than themselves (Fig. 1).

Mating

After assisting the female to molt the male crab releases the cast exuvium and reclasps his soft partner in the same manner as before. He inverts her beneath his body so that their abdomens are in close proximity and proceeds to spread spermatophore bands over her pleopods. Ejaculation is accomplished

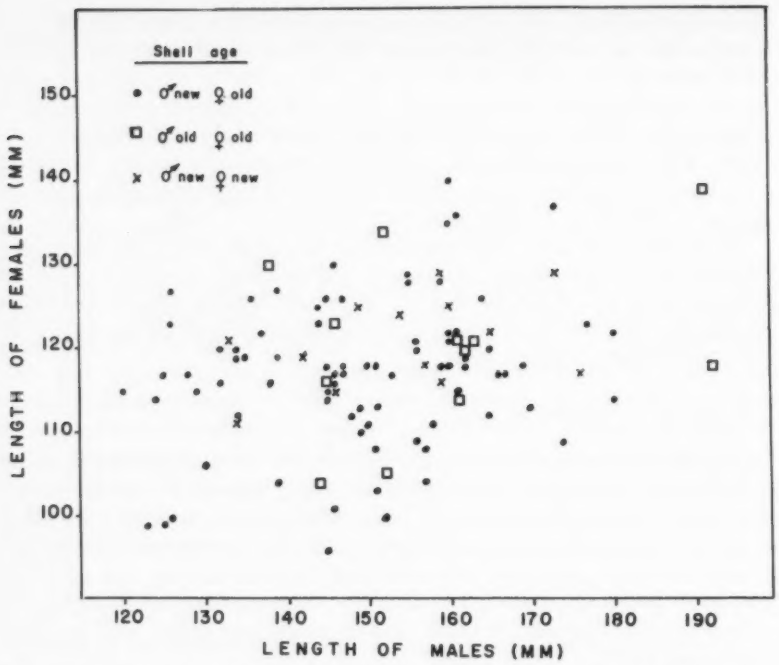

FIG. 1. Carapace-length shell-age relationship of 106 breeding pairs of grasping male and female king crabs captured by divers.

by extending the fifth pair of periopods from beneath his carapace and moving them vigorously amongst the pleopods of the female's flexed-open abdomen. Spermatophore bands issue from the genital pores at the basipodites of the fifth periopods. Ovulation usually commences during this procedure, and at its completion the male leaves the female. Eggs flow from the genital pores located at the ventral surface of the coxopodites of the third periopods and become attached to the many hairs of the pleopods, where they develop and hatch 11 months later. Kurata (MS, 1961) states that ejaculation and ovulation normally are completed within 2 hr after the female molts.

The reproductive period of the lobster (*Homarus americanus*) has been described by Herrick (1902). Unlike the lobster, female king crabs have no seminal receptacle, and therefore, require the presence of a male during ovulation. Kurata (MS, 1961) states that successful spawning did not occur among females which were kept separate from males for the first 5 days after ecdysis. Although mating occurred, the eggs did not adhere to the pleopodal hairs, but dispersed in the water.

The inability of old-shell females to oviposit has led to the suppositions that the genital pore may be too rigid to permit extrusion of eggs, or that the physiology of molting necessarily precedes ovulation, or a combination of

these factors. During an observation of a male grasping a swelling pre-molt female, a spermatophore band was spread over the pleopods of her flexed-open abdomen and the simulation of mating was apparent. This may suggest that while the female was physiologically prepared for breeding a mechanical rather than biological impediment existed which restricted ovulation.

METHODS

One hundred and six grasping pairs of king crabs were captured by scuba-equipped biologists on numerous dives during the late winter and early spring of 1963 and 1964 (Table I). Although many pairs were observed only half of them were brought to the surface and placed in a skiff for transport to Kodiak, or to allow measurements to be taken. Most of the males retained a firm hold on their female partners, though out of water for as long as 1 hr. Exoskeletal ages were determined according to their appearance by accepted procedure (Weber and Miyahara, 1960); carapace measurements were recorded to the nearest millimeter with vernier calipers; and crabs destined for further study were marked by tying colored plastic to their legs.

On March 24 and 25, 1964, five of the grasping pairs were collected at 7 fathoms depth from the reefs of Near and Puffin Islands, just seaward from Kodiak village. Of the five males, one sublegal and two legal were placed in a 4 ft × 4 ft × 6 ft glass aquarium with the five females for breeding observations. The other two males were returned to the sea. Males ranged in length from 120 to 152 mm and were selected so that the largest and smallest were new-shells, estimated to have molted in February. The third was an anexuviant, 144 mm in length, and had not molted for about 13 months.

The second aquarium study was conducted in the 12 ft × 16 ft × 25 ft holding tank of the 85-ft crab fishing boat, *Sea Quail*. During April 15 and 16, 1964, 11 sublegal, new-shell male graspers and 36 adult female graspees were placed in the tank, and on April 21, 15 additional adult female graspees were added. Males which were separated from females and not used in the experiment were returned to the water. Depth of capture was 3–8 fathoms, and locations of capture were the reefs of Gull, Near, and Puffin Islands. The hatch of the tank was open throughout the experiment thus supplying a natural source of light. Divers used a 1000-w underwater lamp during observations of 2 hr each day. Crabs of the first study were observed for 4 days before they were destroyed by the March 27, 1964, tidal wave. The second study began April 15, 1964, and lasted 16 days. Records were kept of the number of females grasped and mated by each male, and time intervals between swelling and molting of the females.

Growth of newly-molted females and samples of their eggs were obtained periodically. Eggs were placed in glass vials of Bouin's solution for 3–6 hr to allow for fixation and hardening, and microscopic examination determined if they were fertilized. Attempts at using Smith's Modification of Bouins solution proved unsatisfactory, because the eggs became opaque, making obser-

TABLE I. Relationship of carapace size and exoskeletal age between partners for 106 breed... and April 1964, Cha...

Date captured	Grasping pair number	Male crabs			Female crabs		
		Carapace		Exoskeleton age (mo.)	Carapace		Exoskele... age (mo...
		Length	Width[a]		Length	Width	
(*mo/day/yr*)							
3/25/64	1	120	–	2	115	–	12
3/26/64	2	123	–	3	109	–	12
4/16/64	3	124	137	3	114	122	12
4/15/64	4	125	143	1	109	114	12
4/16/64	5	125	139	2	117	125	12
4/16/64	6	126	141	3	127	134	12
3/26/64	7	126	–	2	100	–	12
4/21/64	8	126	143	2	123	137	molting
4/21/64	9	128	153	2	117	128	12
4/21/64	10	129	115	2	115	126	12[c]
3/26/64	11	130	–	2	106	–	12
4/16/64	12	132	152	2	116	125	12
4/15/64	13	132	160	1	120	134	12
4/21/64	14	133	153	2	121	133	2
4/21/64	15	134	154	3	112	120	12
4/21/64	16	134	155	2	119	129	12
4/16/64	17	134	156	1	120	127	12[c]
4/15/64	18	134	158	1	111	123	1
4/16/64	19	135	161	1	119	129	12
4/21/64	20	136	152	2	126	136	12
4/21/64	21	137	159	2	122	131	12
4/ 7/63	22	138	161	1	116	122	12
4/ 7/63	23	138	162	12	130	136	12
4/16/64	24	138	163	2	116	128	12[c]
3/26/64	25	139	–	3	104	–	12
4/16/64	26	139	160	1	119	129	12
4/ 7/63	27	139	160	3	127	132	12
4/ 7/63	28	142	167	1	119	132	1
4/16/64	29	144	166	1	125	138	12
3/24/64	30	144	–	12	104	–	12[c]
4/21/64	31	144	166	2	123	129	12[c]
3/26/64	32	145	–	3	96	–	12
3/26/64	33	145	–	1	118	–	12
4/16/64	34	145	168	1	114	122	12
4/21/64	35	145	169	13	116	124	12[c]
4/ 7/64	36	145	170	1	126	142	12
4/16/64	37	145	172	2	115	127	12[c]
4/ 7/63	38	146	168	1	115	131	1
4/16/64	39	146	171	13	123	135	12
4/21/64	40	146	171	1	117	128	12
4/ 7/63	41	146	173	1	116	125	12
4/16/64	42	146	175	1	101	106	12
4/16/64	43	146	176	1	130	144	12
4/21/64	44	147	168	2	117	130	12
4/ 7/63	45	147	171	1	118	126	12
4/21/64	46	147	174	1	126	130	12[c]
4/16/64	47	148	174	2	112	124	12[c]
3/25/64	48	149	–	2	110	–	12
4/ 7/63	49	149	171	1	113	123	12[c]
4/15/64	50	149	172	1	125	140	1
4/21/64	51	150	175	2	118	132	12
4/ 7/63	52	150	176	1	111	120	12
3/26/64	53	151	–	5	113	–	12

[a]Biological investigations utilize the more accurate inside-the-spine carapace width measurements,
[b]This male crab was observed spreading spermatophore bands upon the swimmeret...
[c]Beginning of ecdysis indicated by swelling of the abdomen.

...rs of king crabs taken from the natural environment by scuba divers April 1963, March 1964, ..., Kodiak, Alaska.

	Date captured	Grasping pair number	Male crabs			Female crabs		
			Carapace		Exoskeloton age (mo.)	Carapace		Exoskeloton age (mo.)
			Length	Width[a]		Length	Width	
	day/yr							
12	3/26/64	54	151	–	4	103	–	12
12	4/15/64	55	151	178	1	118	130	12
12	4/ 7/63	56	151	182	1	108	118	12
12	3/25/64	57	152	–	12	105	–	12
12	3/24/64	58	152	–	3	100	–	12
12	4/16/64	59	152	177	15	134	149	12[c]
12	4/16/64	60	153	172	1	117	127	12[c]
olting	4/21/64	61	154	174	1	124	131	3 days
12	4/21/64	62	155	178	1	129	143	12
12[c]	4/15/64	63	155	185	1	128	139	12
12	4/ 7/63	64	156	181	1	109	122	12
12	4/21/64	65	156	182	1	121	130	12
12	4/16/64	66	156	184	1	120	127	12
2 da	3/26/64	67	157	–	2	104	–	12
12	4/ 7/63	68	157	172	1	118	130	1 day
12	4/15/64	69	157	185	1	108	119	12
12[c]	4/ 7/63	70	158	189	1	111	122	12
1 da	4/ 7/63	71	159	181	1	116	127	1 day
12	4/ 7/63	72[b]	159	183	1	129	136	1 day
12	4/16/64	73	159	184	1	128	139	12
12	4/21/64	74	159	189	2	118	130	12
12	4/16/64	75	160	184	1	121	130	12
12	4/21/64	76	160	185	1	140	155	12
12[c]	4/15/64	77	160	187	1	135	147	12
12	4/ 7/63	78	160	188	1	118	126	12[c]
12	4/21/64	79	160	189	1	122	133	12
12	4/21/64	80	160	190	2	125	139	1 day
1 da	4/16/64	81	161	187	1	115	125	12[c]
12	4/ 7/63	82	161	189	1	122	132	12
12[c]	4/ 7/63	83	161	190	12	114	124	12[c]
12[c]	4/21/64	84	161	190	1	136	149	12
12	4/15/64	85	161	190	11	121	130	12
12	4/15/64	86	162	188	1	118	127	12
12	4/ 7/63	87	162	189	1	119	126	12
12[c]	4/ 7/63	88	162	191	12	120	126	12
12	4/15/64	89	163	190	13	121	132	12
12[c]	4/16/64	90	164	194	1	126	136	12
1 da	4/ 7/63	91	165	189	1	120	130	12
12	4/ 7/63	92	165	194	1	112	121	12
12	4/21/64	93	165	196	1	122	133	1 day
12	4/15/64	94	166	191	1	117	127	12
12	4/ 7/63	95	167	185	1	117	127	12
12	4/21/64	96	169	198	2	118	133	12
12	4/ 7/63	97	170	201	2	113	123	12
12	4/21/64	98	173	205	1	129	142	1 day
12[c]	4/16/64	99	173	206	1	137	146	12
12[c]	4/ 7/63	100	174	204	1	109	119	12
12	4/ 7/63	101	176	202	1	117	123	1 day
12[c]	4/ 7/63	102	177	210	1	123	132	12
1 da	4/ 7/63	103	180	211	1	122	131	12[c]
12	4/ 7/63	104	180	214	1	114	125	12
12	4/30/64	105	191	229	14	139	151	12
12	4/16/64	106	192	222	14	118	129	12

...h measurements, which differ about 5 mm from the commercial method of measuring outside the spines.
...mere...med under female partner.

vations difficult. Eggs were preserved by transferring them from Bouins to 70% ethyl alcohol containing glycerin.

At the termination of the investigation, the third pleopod of eggs was removed from each female and preserved in large jars for future reference. Reproductive tracts of the males were removed and studied microscopically to determine if mature sperms were present. Sections of the ductus deferens were split open to expose the spermatophore band, which in turn was sectioned, and prepared on slides. Powers of magnification used were: 10 × .25, 43 × .66, 97 × 1.25. Bouins solution and 70% glycerated ethyl alcohol, respectively, were used to fix and preserve the specimens. Eggs fixed in Bouins solution would not stain in borax carmine, so they were unsuccessfully mounted on glass slides. Only those eggs which were placed directly in 70% alcoholic-acetic fixation without immersion in Bouins solution resulted in good mounts.

OBSERVATIONS ON SPAWNING AREAS

Description of Spawning Areas

Breeding king crabs and those in the precopulatory embrace, which were observed during 1963 and 1964, occupied the subtidal peripheries of kelp-covered reefs approximately ½ mile seaward from the town of Kodiak, Alaska. Crabs were concentrated at depths of 3–8 fathoms within the lower zone of kelp and boulders. The zones varied in width from 15 to 200 ft depending on the slope after which they merged with a level expanse of brown mud, or shell-sand substrate. Divers searched deeper beyond the shell-sand and onto the mud, but found few breeding crabs. Spawning crabs appeared to prefer the kelp areas where *Alaria*, *Costaria*, and *Laminaria* were common. The rocks and kelp probably afford protection to the soft female during ecdysis, and subsequent mating.

No solitary females and few solitary males were seen on the breeding grounds, suggesting that mated crabs soon depart from the reefs after breeding and that pairing occurs in deeper, adjacent water. The few grasping pairs found in deeper water free from kelp and rock may have been traveling to the breeding areas.

Size and Shell Condition of the 106 Grasping Pairs

The carapace length of embracing male and female crabs, respectively, ranged from 120 to 192 mm, and 96 to 140 mm (Table I).

Of the 106 males, 67 (63%) were sublegal. Only 6 (9%) of the sublegal males were anexuviants whose exoskeletal ages ranged from 12 to 15 months, and the remaining 61 (91%) were recently molted new-shells. Of the 39 legal males, 6 (15%) were anexuviants with shell ages of 11–14 months, and 33 (85%) were new-shells.

Of the female graspees, 13 (12%) had recently molted and 93 (88%) were old-shells preparing for ecdysis. A few of the old-shelled females still had small numbers of unhatched eggs attached to the pleopodal hairs; 16

(17%) of the old-shell females were swelling prior to exuviation; and one female was in the process of molting.

RESULTS OF STUDIES IN ARTIFICIAL ENVIRONMENTS

Glass Aquarium

The beginning of ecdysis is apparent by the swelling of the abdomen as a result of increased water intake, and eyes becoming bright red. The abdomen of one of the five females was swollen at the time of capture, molting occurred 7½ hr later. Another female molted 6 hr after swelling. At the time the tidal wave destroyed the experiment two others had been swelling for 24 hr, the last had not yet begun.

The anexuviant male mated with both post-molt females and was grasping a third when the experiment was ended. The new-shell males each grasped various partners, but since molting did not occur they were not observed to mate. Unmated females appeared to be congregated around the largest new-shell male. The females were relatively immobile before and during ecdysis and were completely submissive to the males.

Considerable fighting occurred among grasping males usually resulting in partner exchange. Fighting was most prevalent when pairs came in close proximity with one another, or when females were removed creating a ratio of more males than females. Fighting was not observed in the natural environment.

The only feeding observed was that of the old-shell male which ate a piece of dead herring after mating with a female. Shed exuvia, starfish, and sea urchins were available but not eaten.

Steel Tank in Boat

During April 15 to 24, 1964, the 11 males mated with the 51 females, and subsequent fertilization was determined to be 100% successful by microscopic examination of the ova. Thus, each male mated with an average of 4.6 females, although a range for graspers of 2–8 females per male was observed. The experiment was not terminated until April 30, 1964, in order to allow for superficial cleavage of eggs. Mr. T. Hanoaka (Fisheries Agency, Japan, personal communication) states that a male with a carapace length of 133 mm successfully mated with five females, but two following attempts were abnormal: "... there were mating movements but the male could not release sperm and there were no releasing of new eggs from the female ..." A second male with a carapace length of 125 mm "... mated normally with the first 3 females and also again abnormally with the ... (fourth)." Unfortunately, exoskeletal ages of breeding males were not provided.

Near the completion of the present investigation a full clutch of eggs was present on all but one of the 51 females. Upon dissection of the female with the 10% clutch, each oviduct was found to be full of developing eggs. Most of the ova were white, but a few brown ones were interspersed among

them. Field investigations of internal eggs of adult females prior to and following ovulation revealed that the eggs in question were developing for the next spawning season and that the partial clutch was therefore due to lack of egg adherence to the pleopodal hairs after ovulation. This would suggest the lack of a fully capable male, which is substantiated from work done by Kurata (MS, 1961). Examination of the ductus deferens revealed an abundance of mature sperms in all 11 male crabs.

Female ecdysis required 6–36 hr from the commencement of swelling. Increase in female exoskeletal size corresponds to data collected by Powell (MS, 1960) and Gray (MS, 1963).

Limited attempts were made to study feeding prior to, during, or after breeding by introducing pieces of herring, live starfish, and exuvia into the tank. None of the breeders was observed ingesting food materials.

SUMMARY AND CONCLUSIONS

Crabs form pairs prior to mating whereupon females molt into breeding form. No relationship between size of partners was evident among 106 grasping pairs captured by divers.

Females must molt and become "soft shell" to ovulate, but males can breed as new-shells or as anexuviants.

Although divers found king crabs congregating on rocky kelp-covered reefs from 3 to 8 fathoms, it is possible that they also breed in deeper water, on mud, or on shell-sand substrates.

Eighty nine per cent of the 106 breeding males observed in nature in late winter and early spring were new-shell, indicating they had molted within the previous 3 months.

Eleven sublegal, recently-molted males serviced 51 adult females indicating that young males may suffice as brood stock if large males are cropped by commercial fishermen.

Breeding crabs were abundant and seemingly unaffected by the tidal wave which destroyed Kodiak on March 27, 1964.

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Activity Coefficient of the Magnesium Ion in Sea Water[1]

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ABSTRACT

Sodium hydroxide was added to carbonate-free sea water and the point at which $Mg(OH)_2$ precipitated was detected optically. At this point the hydroxyl ion activity was calculated from the measured pH of the solution and the magnesium ion activity was calculated from the activity product for $Mg(OH)_2$.

From a knowledge of the magnesium ion activity and other data, it was estimated that about 3% of the total magnesium in the sea water was in the form of $MgSO_4^\circ$.

INTRODUCTION

The measurement of activity coefficients of ions in sea water is essential to the estimation of extent of inorganic complexing in sea water. For example, association constants are generally expressed in terms of activities, $a_i = \gamma_i m_i$, where γ_i is the activity coefficient of species i and m_i is its molal concentration. In very dilute solutions γ_i approaches unity, and equals unity in infinitely dilute solutions, but in sea water it can be very much less than this for some ions. It is possible to estimate γ_i in sea water by various empirical means (Sillén, 1961), but generally the values must be measured experimentally.

If a base is added to sea water containing no carbonate ions, magnesium hydroxide will be precipitated when its activity product has been exceeded (Ishizaka and Wada, 1957). At this point $a_{Mg^{++}} \times a^2_{OH^-} = K_o$, the activity product for magnesium hydroxide. Since the pH of a solution is $-\log a_{H^+}$ we can calculate a_{OH^-} from the ionization constant for water, $K_w = a_{H^+} \times a_{OH^-}$, and knowing the magnesium molality in sea water, $m_{Mg^{++}}$, we can then calculate

$$\gamma_{Mg^{++}} = a_{Mg^{++}}/m_{Mg^{++}}$$

or

$$\gamma_{Mg^{++}} = \frac{K_o}{K_w^2} \times \frac{a^2_{H^+}}{m_{Mg^{++}}} \qquad \ldots\ldots (1)$$

The initial precipitation point can be measured by a very sensitive light scattering photometer (Platford, 1964).

EXPERIMENTAL

All chemicals were A.R. grade.

The pH meter was a Radiometer Model 4 with type B glass electrode (low salt error) and a saturated calomel reference electrode. It was calibrated with the following buffers:

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$0.05M$	$Na_2B_4O_7$	pH 9.18 at 25°C
$0.05M$	$NaHCO_3 + 0.05M$ Na_2CO_3	pH 9.93 at 25°C
$0.01M$	$NaHCO_3 + 0.01M$ Na_2CO_3	pH 10.11 at 25°C

Turbidities were measured with a Brice Phoenix 1000 Series light scattering photometer with a constant temperature jacket surrounding the scattering cell.

All runs were made at 25 ± 0.2°C.

The method consisted of adding small increments of $0.01M$ NaOH to a synthetic sea water and measuring the turbidity and pH after each addition. The sea water had the following composition:

$0.4240M$	NaCl
$0.0553M$	$MgCl_2$
$0.0291M$	Na_2SO_4
$0.0105M$	$CaCl_2$
$0.0094M$	KCl

This water had a salinity of 35‰ and was very similar to natural sea water except that it lacked some minor constituents, including carbonate which would have precipitated $CaCO_3$ on addition of base. Trace constituents would not affect γ_i of a major ion significantly. Lower salinities were prepared by dilution with distilled water.

A plot of turbidity as a function of pH revealed an inflection point attributed to precipitation of $Mg(OH)_2$ as shown in Fig. 1. The value for $\gamma_{Mg^{++}}$ was calculated from equation (1) where:

$K_o = 10^{-10.74 \pm 0.17}$ and $K_w = 10^{-14.00}$ (Bjerrum *et al.*, 1958).

TABLE I. Precipitation points and activity coefficients of Mg^{++} in sea water at 25°C.

Salinity	Precipitation pH	$a_{Mg^{++}}$	$m_{Mg^{++}}$	$\gamma_{Mg^{++}}$
5‰	10.07 ± 0.03	1.32×10^{-3}	7.70×10^{-3}	0.17 ± 0.06
15‰	10.00 ± 0.03	1.82×10^{-3}	23.2×10^{-3}	0.078 ± 0.03
25‰	9.92 ± 0.03	2.63×10^{-3}	39.1×10^{-3}	0.067 ± 0.03
35‰	9.86 ± 0.03	3.46×10^{-3}	55.3×10^{-3}	0.063 ± 0.03

In Table I are given the pH of initial precipitation, $a_{Mg^{++}}$ $m_{Mg^{++}}$ and $\gamma_{Mg^{++}}$ as a function of salinity. The results are plotted in Fig. 2. Each result is the mean of at least five determinations and is shown with its average error. Because of the relatively large uncertainty in K_o the absolute values of these results are only good to about $\pm 40\%$ although the relative values are good to about $\pm 15\%$. It was assumed that the reaction occurring at the precipitation point was (Heubel, 1949):

$$Mg^{++} + 2OH^- \rightarrow Mg(OH)_2$$

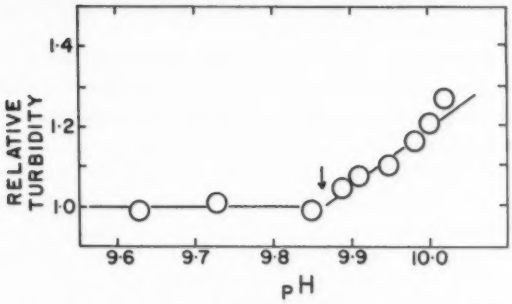

FIG. 1. Typical plot of turbidity as a function of pH for 35‰ sea water at 25°C.

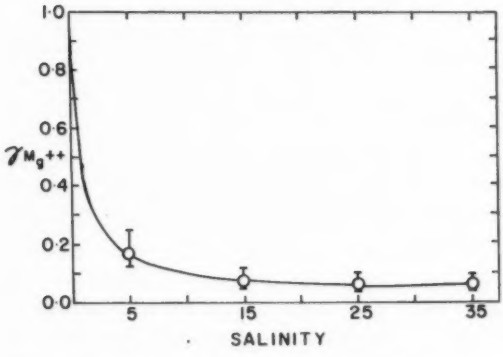

FIG. 2. Activity coefficients of the magnesium ion in sea water at 25°C.

and experiments with dilute $MgCl_2$ solutions supported this assumption. Since about 0.3% of the magnesium was in the form of $MgOH^+$ at the precipitation pH (Davies, 1962), the reaction:

$$MgOH^+ + OH^- \rightarrow Mg(OH)_2$$

was of minor importance.

DISCUSSION

It should be pointed out that single ion activity coefficients depend on EMF measurements made with a liquid junction and that such measurements are subject to an error due to the unknown EMF developed across this junction. For example, pH measurements are based on the assumption that the junction potential of the reference electrode salt bridge is the same for the calibrating buffer solution as it is for the solution whose pH is being measured. This is not always true, especially in sea water, so this limitation on single ion activity coefficients should be recognized at the outset.

It should also be noted that any redeterminations of K_o for magnesium hydroxide will change $\gamma_{Mg^{++}}$ accordingly.

Garrels and Thompson (1962) have reported a value of 0.36 for $\gamma_{Mg^{++}}$ in sea water. However, their value, which was obtained by calculation rather than measurement, involved entirely different assumptions than those made in the present work.

The only anion in sea water which Mg^{++} might be expected to react with is the sulfate ion and Davies (1962) has given the equilibrium constant for the reaction as:

$$\frac{a_{MgSO_4^0}}{a_{Mg^{++}} \times a_{SO_4^{--}}} = 170.$$

Unpublished work from this laboratory indicates a value for $\gamma_{SO_4^{--}}$ of about 0.1 so that for 35‰ sea water at 25°C we can calculate:

$$m_{MgSO_4^0} = \frac{170 \times m_{Mg^{++}} \times \gamma_{Mg^{++}} \times m_{SO_4^{--}} \times \gamma_{SO_4^{--}}}{\gamma_{MgSO_4^0}}$$

$$m_{MgSO_4^0} \simeq 1.7 \times 10^{-3}$$

if we assume $\gamma_{MgSO_4^0}$ (an uncharged species) is unity. Thus, about 3% of the Mg in sea water is tied up as the $MgSO_4^0$ complex. No other magnesium complexes should be formed in sea water — at a pH of 8, $MgOH^+$ would account for less than 0.01% of the Mg.

It is interesting to compare these results with some values of Wattenberg and Timmerman (1938) who found $K_o = 10^{-11.0}$ and who gave a value of $10^{-10.3}$ for the solubility product of $Mg(OH)_2$ in sea water. The present work indicates a value considerably larger — $m_{Mg^{++}} \times a^2_{OH^-} = 10^{-9.54}$. Ishizaka and Wada (1957) found $m_{Mg^{++}} \times a^2_{OH^-} = 10^{-9.48}$.

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Tetracycline Antibiotics in Shrimp Preservation[1]

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ABSTRACT

Certain bacteria isolated from raw and cooked shrimp displayed reduced sensitivity to tetracycline antibiotics in the presence of multivalent metallic cations or shrimp extracts; addition of a chelating agent resulted in reduction of the antagonism of such ions and extracts toward the antibacterial action of these antibiotics. In shrimp preservation, a dip in the chelating agent increased the efficacy of a subsequent tetracycline antibiotic dip when the product was judged by organoleptic observation or by viable bacterial counts.

INTRODUCTION

During the course of investigations concerned with the use of tetracycline antibiotics for preservation of fishery products, it was found that the level of chlortetracycline efficient for use with fish was generally not effective in retarding bacterial spoilage of shellfish (Tarr *et al.*, 1952; Vance, 1959; Tarr, 1961).

It has been shown that the antibacterial activity of the tetracycline antibiotics is considerably reduced in the presence of divalent cations (Weinberg, 1954, 1957; Hamburger, 1957; Price, 1957); the fact that shellfish normally have a higher concentration of Mg^{++} and Ca^{++} than do fish (Tarr, 1955) offers a possible explanation for the difficulties encountered in the use of tetracycline antibiotics for shellfish preservation.

Southcott and Tarr (1961) reported that the sensitivity of various bacterial isolates from Pacific coast dogfish to four of the tetracycline antibiotics was greater on nutrient agar than on sea water agar; when these isolates were tested on a synthetic agar, increasing the concentration of Mg^{++} reduced the sensitivity of the isolates. Castell and Dale (1963) showed that a number of multivalent cations would react with chlortetracycline in buffered solutions with attendant decrease in the bacteriostatic activity of the antibiotic. In tests involving the addition of such ions to antibiotic-treated cod fillets, a reduction in keeping quality was reported.

In the work described here, aerobic bacteria isolated from the surface of shrimp were studied in order to determine their sensitivities to tetracycline antibiotics; the effect of metallic cations and of shrimp extracts upon this sensitivity was investigated. Bacteria with widely differing characteristics were tested to ascertain whether any one type of bacterium present on the surfaces of commercially processed shrimp could be responsible for the relative ineffectiveness of tetracycline antibiotics in shrimp preservation when the latter were applied at the levels recommended for use in preservation of fish. The presence

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of a chelating agent was shown to modify the effect of multivalent cations as inhibitors of the antibiotic activity of tetracyclines against bacteria from shrimp. Results of preservation tests with raw shrimp indicated that a dip in a chelating solution increased the efficiency of a subsequent antibiotic dip.

MATERIALS AND METHODS

Isolation and Characterization of Bacteria

All media used were adjusted to pH 6.8.

Commercially cooked, headed, peeled shrimp, and raw, headed, unpeeled shrimp were smeared on the surface of hardened plates of BBL Eugonagar (EA) which were then incubated at 25°C for 48 hr. Colonies of apparently different bacteria were picked onto EA plates and after further growth, and purification when necessary, were kept as stock cultures on EA slants. The usual bacteriological techniques and media were employed in attempting to classify the organisms (Anon., 1957; Conn, 1957; Skerman, 1959).

Growth studies revealed that the majority of these bacteria from shrimp did not grow quickly or abundantly on the synthetic agar (Southcott and Tarr, 1961) previously employed with other organisms for testing cation effects; for this reason EA, nutrient agar (NA), and sea water agar (SWA) (Southcott *et al.*, 1960) were used.

Sensitivity tests on the bacteria were carried out using Bacto Sensitivity Discs (10 μg strength) of four tetracycline antibiotics [tetracycline (TC), chlortetracycline (CTC), oxytetracycline (OTC), and demethylchlortetracycline (DCTC)] on inoculated hardened plates of NA, SWA, or EA. The zones of inhibition were measured using a Fisher-Lilly Antibiotic Zone Reader.

Gradient plates (Szybalski and Bryson, 1952) were prepared, incorporating a metallic cation in the lower layer and an antibiotic in the top layer of two-layer EA plates.

In some cases, where the growth was thin and the same colour as the agar, difficulty was experienced in determining the exact edge of zones of inhibition on sensitivity disc plates and on gradient plates. To overcome this problem, Whatman filter paper circles of a diameter equal to that of a Petri dish bottom were dipped in a 2% solution of triphenyl tetrazolium chloride, drained briefly, then applied to the agar surface of the plate, care being taken to eliminate air bubbles. The development of a deep red-purple colour on the paper indicated the presence of growth in the corresponding area of the agar; zones of inhibition could be conveniently measured with or without removal of the paper.

Shrimp Extracts

Water extracts of meat or shell portions of raw or cooked shrimp were used in certain sensitivity tests. Shrimp meat or shell was blended with an equal weight of water, autoclaved at 15 psi for 15 min, filtered through glass wool, reduced to half volume by heating on a hot plate at 98°C, adjusted to pH 6.8 and sterilized.

The same procedure was used to prepare a water extract of cod fillets for comparative purposes.

Shrimp Agar

Shrimp agar was prepared by introducing pieces of chopped shrimp meat (5–10 g per 20 ml agar) into flasks of EA before sterilization. The neck of each flask was covered with a piece of fine cheesecloth and a protective inverted beaker. After sterilization, the agar was poured through the cheesecloth, which retained the pieces of meat.

Cod agar was similarly prepared using chopped cod fillets.

For some tests, shrimp agar was prepared by the addition of EA powder to a water extract of shrimp meat or shrimp shell.

Shrimp Preservation Tests

Raw, unpeeled, male shrimp, unsorted as to species, were obtained, whole or headed as required, from a commercial shellfish company. The shrimp were washed at the laboratory, then dipped for 2 min in the appropriate solutions of Sequestrene (SEQ) (disodium ethylenediamine tetraacetate dihydrate, Food Grade — Geigy Chemical Corp.) or antibiotic (CTC, Cyanamid of Canada; OTC, Chas. Pfizer Co.) and drained 3 min on wire racks; control batches were dipped in water. The pHs of dip solutions were between 4.6 and 5.6. Where there was a double dip, shrimp were drained 3 min between dips. The shrimp were loosely packed into polyethylene bags and stored at 0°C.

At intervals, samples were removed for organoleptic observation and viable bacterial counts. The counting method comprised the shaking and plating of a 40-g sample of shrimp according to the method of Boyd and Southcott (1962).

RESULTS AND DISCUSSION

The morphological and biochemical characteristics of some of the different bacteria isolated from shrimp are presented in Table I. All the isolates were aerobic. No spores were detected in any culture by staining; transfer of heat-shocked cells produced no growth. No isolate hydrolyzed starch, utilized lactose, produced indole, formed hydrogen sulphide, or grew with $NH_4H_2PO_4$ as the sole nitrogen source.

Gram-positive (G+) cocci were isolated from both raw and cooked shrimp; they may have originated from the marine environment or as a result of handling procedures. In this group, no culture showed consistent formation of regular packets, but tetrads were often seen. Baird-Parker (1963) has stated that the ability of cocci to form cubical packets appeared to be a stable character while formation of loose or compact clusters showed considerable variation on different media. Three of the cocci were oxidase-positive (Steel, 1962), coagulase-negative, and did not ferment glucose. Cultures S1 and S6 had characteristics suggesting their classification in the genus *Staphylococcus* (Table I); however they could not be placed in any species as described in

TABLE I. Characteristics of some bacteria isolated from shrimp.

Isolate	Form	Motility	Flagellar arrangement	Gelatin liquefaction	Action in litmus milk	Nitrate reduction	Methyl red test	Voges-Proskauer test	Catalase	Oxidase	Urease	Growth on NaCl agar: 5%	Growth on NaCl agar: 10%	Growth on NaCl agar: 15%	Glucose oxidation	Glucose fermentation	Mannitol oxidation	Sucrose oxidation	Pigment	Growth: 0°C	Growth: 25°C	Growth: 37°C	Growth: 45°C	Coagulase	Citrate utilization	Aerogenic
G+ rods																										
S2	SP C						+	+	+			+			+	+	+	+		+	+					
S3	SC F	+	P						+			+							Y			+	+	+		
S4	SC F	+	P						+			+							Y			+	+	+		
S7	SP C	+	P						+	+		+										+	+			
S10	S			+	AK D	+			+			+	+						Y	+	+					
S11	S			+	AK D	+			+			+	+						Y	+	+					
16	S			+		+			+			+			+				Y		+	+				
G+ cocci																										
S1	PT B								+		+	+	+	+	+	+	+	+	YO		+	+	+	+		
S6	BP			+		+	+		+			+	+	+	+	+	+	+			+	+		+		
S9	PT								+	+		+	+						O	+	+	+					
S18	PB								+	+		+	+						O	+	+	+					
S20	PB								+	+		+	+						O	+	+	+					
25	PB						+		+		+	+	+		+	+			Y		+	+		+			
109	B				'		+		+		+	+	+		+	+			O		+	+					
G− rods																										
S8	S	+	L	+	RA				+	+		+			+	+				+	+				+		
S12	SC				AK	+			+	+	+	+	+							+	+						
S13	SC				AK	+			+	+	+	+	+							+	+						
S14	S				A	+			+	+		+								+	+						
S16	S	+		+	RD	+		+	+	+		+			+	+	+	+		+	+					+	+

Key: *Form* S = single cells; P = pairs; C = chains; F = filaments; T = tetrads; B = irregular bunches. *Flagella* P = peritrichous; L = lophotrichous. *Litmus milk* R = reduced; A = acid; AK = alkaline; D = digested. *Pigment* Y = yellow; O = orange.

"Bergey's Manual" (Anon., 1957). The other coccus isolates could not be assigned to any species of the genus *Micrococcus*. Anderson (1962), in a study of cocci from the North Sea, proposed a scheme for division of G+, catalase-positive cocci; his groups are not given generic or specific status. Anderson found that fermentative cocci represented 90% of the total of his isolates, and that these fermenters did not all acidify litmus milk. The G+ cocci isolated from shrimp would appear to fit into such a grouping better than into that used in "Bergey's Manual" (Anon., 1957) for coccus forms. The Gram-negative (G–) rods showed a variety of characteristics. Culture S8, extremely motile with lophotrichous flagellae, was found to attack glucose oxidatively or fermentatively and was therefore of the family Pseudomonadaceae, probably genus *Pseudomonas*. Culture S16 showed variable motility, fermentation of glucose with extensive aerogenes is, and certain other reactions typical of the genus *Aeromonas*. Other G– rods were found similar to the genus *Achromobacter*. None of the G– rods could be assigned to species described in "Bergey's Manual" (Anon., 1957) or other conventional classifications.

The total characteristics of the G+ rods (Table I) show the impossibility of their being assigned to any genus in "Bergey's Manual" (Anon., 1957).

TABLE II. Comparative sensitivities of bacterial isolates from shrimp to four tetracycline antibiotics applied as discs on nutrient agar (NA) and on sea water agar (SWA).

Isolate	Zone of inhibition (*mm* diam.)							
	CTC[a]		OTC		DCTC		TC	
	NA	SWA	NA	SWA	NA	SWA	NA	SWA
G+ rods								
S2	19	17	16	11	17	14	18	14
S3	21	20	15	12	19	18	21	18
S4	21	20	17	13	20	18	21	18
S7	22	21	18	12	21	17	21	17
S10	40	22	40	14	40	19	40	19
S11	40	25	30	14	36	19	38	18
16	29	23	24	14	26	19	27	19
G+ cocci								
S1	29	20	24	13	28	16	28	17
S6	31	18	25	11	31	16	31	16
S9	40	27	35	15	42	24	41	23
S18	36	24	30	15	34	20	34	20
S20	33	25	28	16	31	22	32	21
25	35	19	27	13	31	16	31	16
109	33	20	27	13	30	17	31	17
G– rods								
S8	14	0	11	0	15	0	15	0
S12	26	0	20	0	25	0	27	0
S13	29	0	24	0	29	0	29	0
S14	26	0	22	0	25	0	25	0
S16	16	0	13	0	16	0	16	0

[a]CTC = chlortetracycline; OTC = oxytetracycline; DCTC = demethylchlortetracycline; TC = tetracycline.

Cultures S3, S4, and S7 were extremely motile with peritrichous flagellation. Leifson (1964), in studying the motility of marine bacteria, stated that G+ peritrichous flagellate forms were rare in sea water and intestines of fish and shellfish, but common in bottom mud. In the present investigation, the isolates were from shrimp surfaces, which might retain mud, rather than from shrimp viscera.

It is evident that many types of bacteria are represented by this group of cultures from shrimp, and that they cannot be classified by the usual systems. The isolates were studied to determine the effect of the use of tetracycline antibiotics on the different types of bacteria which may be associated with shrimp spoilage.

The comparative sensitivities of the isolates to four tetracycline antibiotics applied as discs on NA and on SWA are given in Table II. Without exception, the shrimp bacteria were less sensitive to tetracycline antibiotics when tested in SWA; the inhibition zone of G– organisms was in every case completely eliminated, whereas G+ organisms remained sensitive to varying degrees. Responses of a G+ rod and a G– rod are again compared in Table III, where NA, SWA, and EA were used; inhibition of the rods by tetracyclines is compared to that by penicillin (P) and streptomycin (S) in the presence of interfering ions present in SWA.

Tests were carried out with isolates of differing characteristics to determine the effect which the presence of certain metallic cations would have upon the sensitivity of the organisms to CTC and OTC when tested in gradient plates.

Isolate 25, a G+ coccus which had been found considerably less sensitive to tetracycline antibiotics in SWA than in NA (Table II), was used in one series of tests. The effects of Mg^{++}, Mn^{++}, Ca^{++} and Fe^{+++} on the response of this organism to 5- and 10-ppm levels of CTC or OTC are given in Fig. 1.

TABLE III. Comparative sensitivities of bacterial isolates from shrimp to tetracyclines (CTC, OTC, DCTC, TC), penicillin (P), and streptomycin (S), applied as discs on nutrient agar (NA), sea water agar (SWA), and eugonagar (EA).

Isolate		Agar	Zone of inhibition (*mm* diam.)					
			CTC	OTC	DCTC	TC	P	S
S3	G+ rod	NA	45	37	40	40	52	16
		SWA	32	15	26	26	52	14
		EA	41	34	38	40	48	17
S7	G+ rod	NA	27	22	25	26	37	19
		SWA	22	10	13	17	41	14
		EA	27	20	24	25	38	21
S13	G– rod	NA	30	23	25	24	0	15
		SWA	0	0	0	0	0	12
		EA	31	27	29	30	0	21

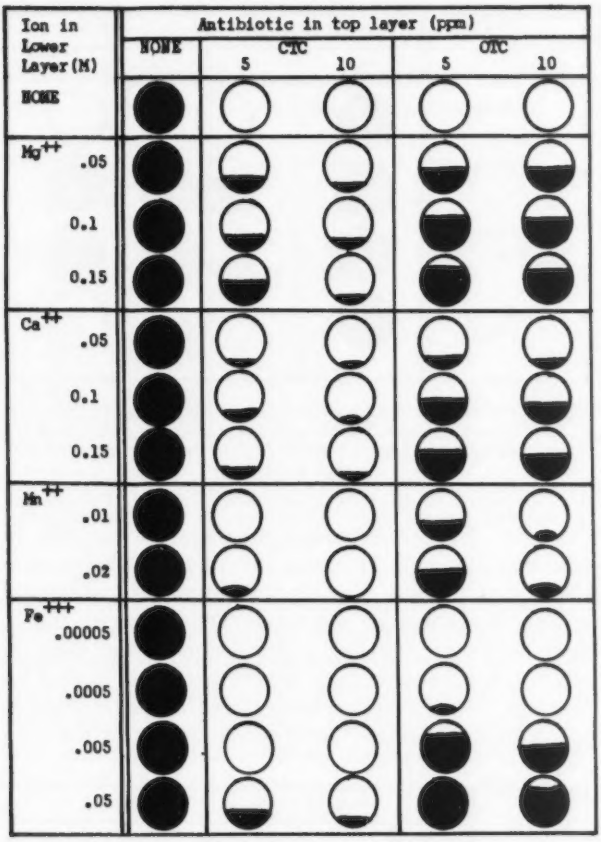

FIG. 1. Sensitivity of a bacterial isolate from shrimp to CTC or OTC on gradient plates containing various cations in the lower layer. Shaded areas indicate growth. Thick edge of lower layer ↓. Thick edge of top layer ↑.

Each of the ions tested interfered to a greater degree with the antibacterial activity of OTC than with that of CTC. When the same procedure was applied to different organisms, the results were similar.

When whole-shrimp agar comprised the lower layer in gradient plates, the antibacterial action of CTC and OTC was modified as shown in Fig. 2; cod agar had no effect. In this case the isolate was a G+ rod (16); tests with other isolates gave the same results. When gradient plates were incubated for extended periods, the bacteriostatic action of tetracyclines was overcome in plates containing Mg^{++} ions or shrimp extract. In some cases, amount of shrimp extract which had not interfered with antibacterial action at 24 or 48 hr allowed, after 96 hr, growth of the organism to a considerably greater

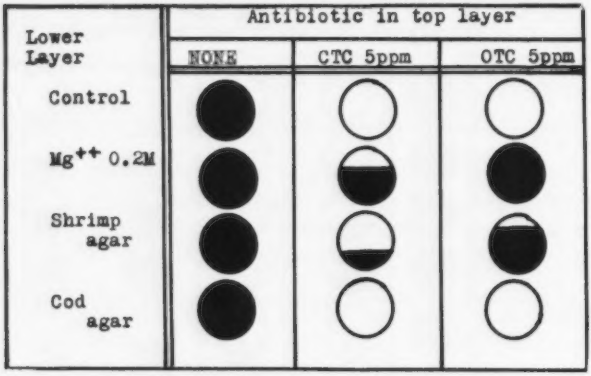

FIG. 2. Sensitivity of a bacterial isolate from shrimp to CTC and OTC on gradient plates containing shrimp or cod agar in the lower layer. Shaded areas indicate growth. Thick edge of lower layer ↓. Thick edge of top layer ↑.

extent than on the antibiotic control plate. Figure 3 presents the results of a typical experiment, using in this case isolate 109 and shrimp-extract agar.

Shrimp-shell extract agar was generally found to antagonize the antibiotic action of tetracyclines to a greater extent than shrimp-meat extract agar; prolonged incubation showed that both meat and shell agars allowed greater growth of test organisms than did the antibiotic control plate (Fig. 4).

In an attempt to determine whether addition of a chelating agent to gradient plates would modify the antagonistic action of shrimp extracts to-

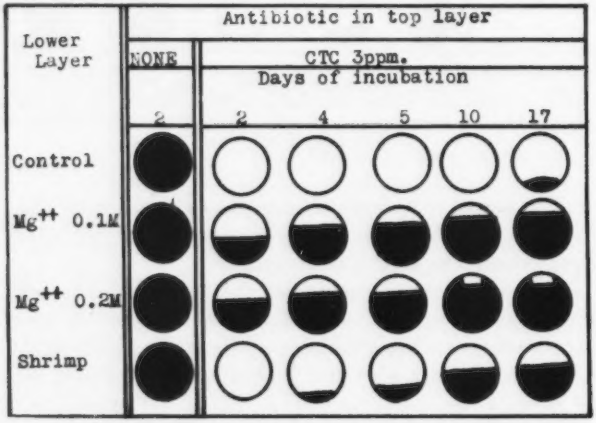

FIG. 3. Sensitivity of a bacterial isolate from shrimp to CTC after prolonged incubation of gradient plates containing shrimp agar in the lower layer. Shaded areas indicate growth. Thick edge of lower layer ↓. Thick edge of top layer ↑.

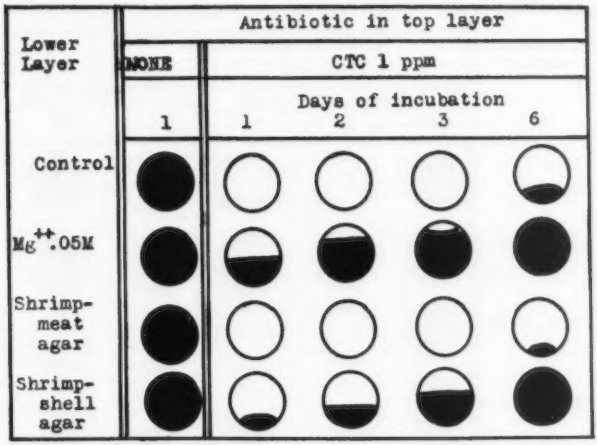

FIG. 4. Sensitivity of a bacterial isolate from shrimp to CTC on gradient plates containing shrimp meat or shrimp shell in the lower layer. Shaded areas indicate growth. Thick edge of lower layer ↓. Thick edge of top layer ↑.

ward tetracycline antibiotics, tests were carried out in which SEQ was added to the lower layer of gradient plates. Results in Fig. 5 are typical of those obtained using isolates of varying characteristics as test organisms.

Sensitivity disc tests were carried out using varying levels of magnesium ion and SEQ in EA single layer plates. Results with several different isolates confirmed that the antagonism between magnesium ions and antibiotic activity could be reversed to varying degrees by SEQ (Table IV).

In Table V, the results of a test involving several G+ rods are presented. Magnesium ions decreased the sensitivity of these organisms to tetracycline antibiotics; SEQ increased the sensitivity by removing some interfering ions present in the control EA plates. When both SEQ and Mg^{++} were present, the sensitivity was restored to substantially what it had been on the control EA plates, especially in the case of CTC.

Since it had been demonstrated by the preceding experiments that in the case of bacterial isolates from shrimp the presence of SEQ increased tetracycline sensitivity, an attempt was made to project the results to the preservation of shrimp. A series of tests was carried out to determine whether treatment of shrimp with SEQ might increase the efficiency of a subsequent tetracycline antibiotic dip in reducing the bacterial load of the shrimp.

In one test (Table VI), the headed raw shrimp obtained were of poor quality, having an initial viable bacterial count of over 6.0×10^6 per gram. In this situation, CTC and OTC dips alone did not affect the further increase of bacterial numbers when compared to the control water dip and only delayed the appearance of putrid and ammoniacal odours by 1 day; however, the shrimp

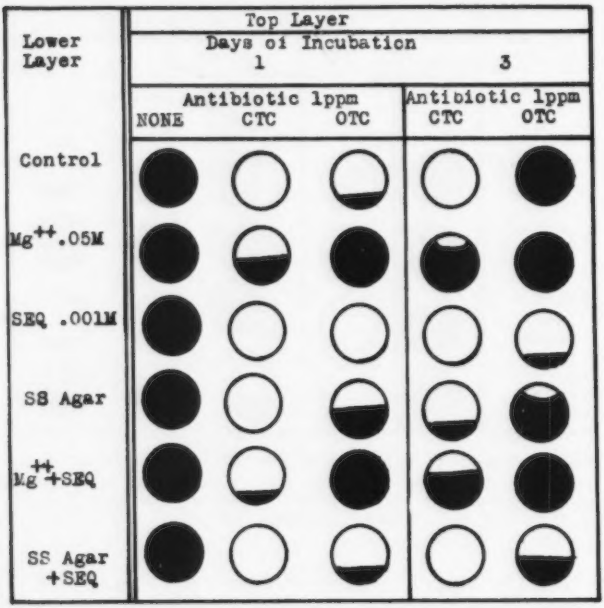

FIG. 5. Sensitivity of a bacterial isolate from shrimp to CTC or OTC on gradient plates as modified by the presence of shrimp shell extract agar (SSA), Mg^{++} or SEQ in the lower layer. Shaded areas indicate growth. Thick edge of lower layer ↓. Thick edge of top layer ↑.

TABLE IV. Sensitivity of a bacterial isolate from shrimp to tetracycline antibiotics (CTC, OTC, DCTC, TC) in the presence of varying levels of Mg^{++} and Sequestrene (SEQ).

Addition to EA	Zone of inhibition (*mm* diam.)			
	CTC	OTC	DCTC	TC
None	27	22	25	24
Mg^{++} .01*M*	23	15	20	20
Mg^{++} .05*M*	20	12	14	13
Mg^{++} 0.1*M*	15	0	10	11
SEQ .002*M*	43	32	38	38
Mg^{++} .01*M* + SEQ .002*M*	31	22	25	27
Mg^{++} .05*M* + SEQ .002*M*	25	14	20	21
Mg^{++} 0.1*M* + SEQ .002*M*	22	9	13	14

TABLE V. Sensitivities of some gram-positive rods to tetracycline antibiotics in the presence of Mg^{++} and Sequestrene (SEQ).

Isolate	Addition to eugonagar	Zone of inhibition (*mm* diam.) CTC	OTC	DCTC	TC
S2	1 None	24	15	21	21
	2 Mg^{++} .02*M*	18	10	14	14
	3 SEQ .001*M*	30	20	27	27
	4 Mg^{++} .02*M* + SEQ .001*M*	24	10	20	19
S3	1	16	11	13	14
	2	14	8	9	11
	3	22	14	18	20
	4	16	9	12	13
S4	1	17	11	15	15
	2	15	8	11	11
	3	22	13	20	20
	4	17	9	13	12
S7	1	16	11	13	14
	2	13	8	10	10
	3	21	14	17	17
	4	15	9	12	11
S10	1	25	18	21	23
	2	19	9	15	16
	3	35	25	32	34
	4	25	15	23	23
16	1	22	15	20	21
	2	19	9	14	15
	3	30	21	28	30
	4	22	11	19	19

which had been dipped in SEQ then in CTC or OTC had lower bacterial counts and were considerably superior when judged organoleptically.

When raw shrimp with heads on were treated and stored, they were organoleptically acceptable after 3 days of storage, but by the fourth day had become extremely putrid with discoloration of the heads. Lantz (1951) reported that removal of the heads extended the storage life of iced raw shrimp. Fieger (1952) has stated that, with Gulf of Mexico shrimp, the heads carry approximately 75% of the bacteria; Duggan and Strasburger (1946) postulated that the putrefactive type of spoilage in shrimp proceeds fairly rapidly when once started. In this experiment, dipping shrimp in antibiotic, even when preceded by SEQ, did not appear to produce a product of better quality than the control; the presence of heads with large bacterial loads probably contributed to the rapid development of putrefactive spoilage after 4 days.

Further tests, using raw headed shrimp of very high quality were carried out. A typical set of results is presented graphically in Fig 6. CTC was used at 15 or 30 ppm and SEQ at 5000 ppm concentrations. Samples which had

TABLE VI. Viable bacterial counts of raw, headed shrimp, dipped and stored at 0°C.

	Bacteria per gram shrimp ($\times 10^6$) Days stored			
Dipping solution	1	3	4	5
Water control	8	110	1620	
SEQ 300 ppm	10	119	1500	
CTC 10 ppm	9	150	1605	3100
OTC 10 ppm	6	145	1355	1645
SEQ 300 ppm + CTC 10 ppm	1	21	115	123
SEQ 300 ppm + OTC 10 ppm	1	20	109	70

been SEQ-dipped developed brown discoloration more slowly than those not so treated, regardless of the antibiotic level used or the viable count of the sample. The samples treated with both SEQ and CTC maintained desirable organoleptic qualities until the eighth day of storage and were in this respect much superior to the samples dipped in SEQ alone, CTC alone, or water.

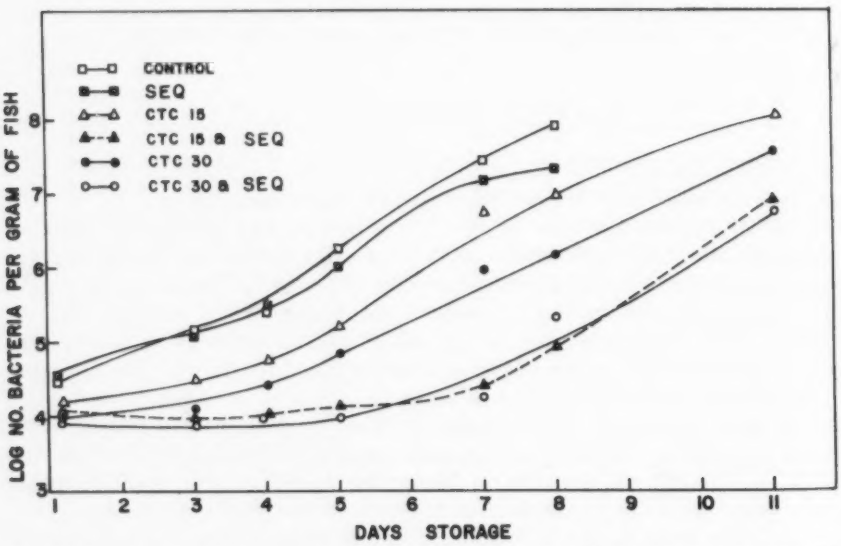

FIG. 6. Viable bacterial counts of raw, headed shrimp dipped in CTC (15 or 30 ppm) and SEQ (Sequestrene 5000 ppm).

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Lipids and Component Fatty Acids of the Newfoundland Squid, *Illex illecebrosus* (Le Sueur)[1]

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ABSTRACT

The component fatty acids of the muscle, liver, and viscera lipids from the squid, *Illex illecebrosus* (Le Sueur), caught in Newfoundland waters have been determined by gas–liquid chromatography (GLC). Silicic acid chromatography was used to segregate the main groups of lipids and the fatty acid composition of each fraction was determined on two organosilicone polyester columns.

The lipids from the muscle consisted mainly of phospholipids with three fatty acids, palmitic (16:0), eicosapentaenoic (20:5), and docosahexaneoic (22:6) acids making up 80% of the total. The liver contained mainly triglycerides with a fatty acid composition similar to liver oils in other marine species.

INTRODUCTION

The squid, *Illex illecebrosus* (Le Sueur), forms the basis for the Newfoundland squid fishery and the catches in recent years have ranged from 5 to 20 million lb. The recent introduction of Japanese fishing methods indicates that the catch could possibly be increased. Most of this is frozen for use as bait, although a considerable amount is utilized fresh.

According to Squires (1957), *Illex* is the only squid found in Newfoundland waters and also occurs as far south as the New England States. The common squid of the New England area is *Loligo pealii* Le Sueur which, as distinct from *Illex*, has a much longer fin relative to its mantle length. *Illex* migrate to Newfoundland in the spring, appearing on the Grand Banks in May–June and along the coast in July–September. Their food at this time consists mainly of small fish, especially capelin, but smaller individuals also feed extensively on euphausiids and other invertebrates. In turn they are the prey of other marine life, particularly pilot whales (Squires, 1957).

To the best of our knowledge, no reports have been published on the properties of the lipids of *Illex* or their component fatty acids. Since most of the catch is frozen and used for bait, the livers are not utilized for oil.

In Japan a large-scale fishery for the squid (cuttlefish), *Ommastrephis solani pacificus*, exists and liver oil is produced commercially. Many of the earlier analyses are now of doubtful value (Adachi, 1960a, b; Takao and Tomiyama, 1953) but recently a good analysis by gas–liquid chromatography (GLC) has appeared (Ito and Fukuzumi, 1963) and some of the highly unsaturated fatty acids have been isolated and identified (Toyama and Takagi, 1962).

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The lipids from the liver of *Octopus dofleini* have been studied (Hatano, 1958a, b, 1961) and a detailed study on the phospholipids of squid and octopus has been published (Zama, 1963). Other molluscs studied (Shimma and Taguchi, 1964; Rodegker and Nevenzel, 1964) have shown a wide variety of fatty acid compositions.

EXPERIMENTAL

A 20-kg block of frozen squid was obtained from Newfoundland and kept at −24°C until used. Fifteen whole squid were thawed and measured and eight of these were used for subsequent lipid extractions. Microscopic analysis of the gonads indicated that they were all females.

The bodies (mantles) and the heads with tentacles were cleaned, wiped dry, and the lipids extracted by the method of Bligh and Dyer (1959). The livers, and the viscera less livers, were macerated in a Waring Blendor with chloroform and sodium sulfate, filtered with the help of Dicalite "Superaid" and the filter cake washed with chloroform. All operations were carried out in a nitrogen atmosphere. Part of the lipids was saponified with KOH–ethanol, the unsaponifiable matter determined according to AOCS Official Methods, and the fatty acids converted to methyl esters using boron trifluoride–methanol (Metcalfe and Schmitz, 1961).

The GLC of the methyl esters was carried out on a Barber-Colman Model 10 with a Model 5121 flame ionization detector, employing two glass columns (3 mm inside diameter, 6 ft in length) packed, respectively, with 15.5% EGSS-Y and 14% EGSS-X organosilicone polyesters on 100–120 mesh Gas-Chrom P[2]. The operating conditions were: column temperature, 200°C, flash heater temperature, 285°C, and argon pressure 15 psi. Provisional identifications of peaks were made by the linear log plot and separation factor procedures (Ackman *et al.*, 1963), and quantification through appropriate correction factors (Ackman and Sipos, 1964).

The column chromatography of the lipids was carried out using 40 g 100-mesh Mallincrodt silicic acid added to a 2.5-cm inside diameter column as a slurry in hexane and packed to a depth of 8 cm. The samples were placed on the column in 50 ml hexane, and the elution was carried out with a slight nitrogen pressure using the following solvent systems: ethyl ether 7%–hexane 93% by (E–H 7:93), chloroform 90%–methanol 10% (C–M 9:1), and methanol 80%–chloroform 20% (M–C 4:1). Two hundred millilitres of each solvent system were used, but since the progress of the elution was not monitored the separations were not as good as if a larger number of fractions had been collected. In addition, the remainder of the flesh lipids (26%) were eluted with 100% methanol followed by methanol 90%–chloroform 10%, and listed as fractions 4 and 5.

Tentative identifications of the lipids in each fraction were made by thin-layer chromatography (TLC). The plates were prepared according to Vogel *et al.* (1962) and developed in the solvent system 69.25–26.5–4.25 of chloroform–methanol–water, respectively. A sample of cod flesh phospholipids was run as a standard. The spots were developed in iodine vapor.

[2]Applied Science Laboratories, Inc., State College, Pa., U.S.A.

The fractions eluted from the column were converted to methyl esters for GLC analysis by the HCl–methanol method of Stoffel *et al.* (1959).

RESULTS AND DISCUSSION

The sizes of the squid were as follows: mantle lengths, "A" (Squires, 1957), 19.5–22.5 cm, average 21.0 cm; fin lengths, "B", 7–8.5 cm, average 8.0 cm; whole weights, 146–206 g, average 173 g, and liver weights, 15.5–27 g, average 23.0 g.

The results of the lipid extraction and the common properties of the lipids are listed in Table I. Except for a slight difference in unsaponifiable matter

TABLE I. Characteristics of lipids from squid.

	Lipids from:			
	Flesh mantle	Flesh head	Liver	Viscera less liver
Per cent lipid	2.0	2.0	22	–
Unsaponifiable matter (%)	12.8	15.1	3.7	11.7
Iodine value of lipids (Wijs)	–	–	190	–
Iodine value of methyl esters (Wijs)	233	220	184	204

between the lipids from the flesh of the body (mantle) and head, the two are very similar. Since the GLC analysis gave essentially the same values for the fatty acid composition of both of these sections, they are listed together as flesh lipids in Table II.

LIVER LIPIDS

The oil from the liver was very dark and became cloudy on standing at temperatures below 25°C. The fatty acids and triglycerides eluted with ether–hexane were colourless and constituted 70% of the total liver lipids. The C–M 9:1 fraction contained one major component also present in cod flesh. This fraction could possibly be cardiolipin or phosphatidic acid. It contained considerably more highly unsaturated fatty acids than the neutral fat fraction, a total of 49% as compared to 28%. The fraction eluted with M–C 4:1 showed traces of lecithin in addition to most of the pigment. Zama (1963) has reported that cuttlefish liver lipids contain 2–3% phospholipids.

The fatty acid composition of the liver oil was very similar to the composition of cuttlefish liver oil as reported by Ito and Fukuzumi (1963) (Table III), and except for a higher proportion of 20:5 and a lower proportion of 22:1, it was also similar to cod liver oil (Ackman and Burgher, 1964a).

FLESH LIPIDS

The lipids from the flesh consisted mainly of phospholipids, and the fatty acid composition was typical for these lipids from marine sources (Ackman and Burgher, 1964b; Schuster *et al.*, 1964).

TABLE II. Silicic acid chromatography of lipids from squid.

	Fraction number	Solvent system	Per cent of total lipid	Tentative identification[a]
Flesh lipids	1	E–H 7:93	3	Neutral lipids, hydrocarbons, sterol esters.
	2	C–M 9:1	37	Phosphatidyl ethanolamine, trace of lecithin, sterols?
	3	C–M 1:4	34	Lecithin, trace of sphingomyelin.
	4	M 100	4	Not identified.
	5	C–M 1:9	22	Not identified.
Liver lipids	1	E–H 7:93	70	Triglycerides, fatty acids 15% sterol esters?
	2	C–M 9:1	27	Phosphatidic acid?, cardiolipin?, sterols, pigment.
	3	C–M 1:4	3	Pigment, trace of lecithin.

[a]In order of abundance.

Fraction 1 from silicic acid chromatography, comprising about 3% of the total, was transesterified and the GLC analysis showed several unknown peaks. Numerous small peaks with retention times less than 16:0 were probably hydrocarbons which are known to occur in marine lipids (Lambertsen and Holman, 1963). A large additional peak with relative retention times of $r = 9.98$ (EGSS-X) and 12.25 (EGSS-Y) relative to methyl oleate, and $r = 0.52$ (EGSS-Y) relative to cholestane, was not identified. Quantification was not attempted due to the many peaks in the region below 16:0.

Fraction 2 eluted with C–M 9:1 had a fatty acid composition as given in Table III. Thin-layer chromatography indicated that this fraction was mainly phosphatidyl ethanolamine with a trace of lecithin. This fraction contained a higher percentage of highly unsaturated fatty acids (59%) than any of the other fractions; Brockerhoff *et al.* (1963) found the same applied to lipids from other marine species. In addition, this fraction contained a high percentage of stearic acid as compared with palmitic acid, confirming the findings of Schuster *et al.* (1964) with regard to the cephalins in tuna phospholipids.

Fraction 3 was eluted with M–C 4:1 and was found by TLC analysis to consist mainly of lecithin with possible traces of sphingomyelin and lysolecithin.

TABLE III. Fatty acid composition of squid lipids in weight per cent (T = trace).

TABLE III. Fatty acid composition of squid lipids in weight per cent (T = trace).

Fatty acids	Double bond position	Liver lipids				Flesh lipids					Viscera lipids	Ito and Fukuzumi cuttlefish oil (liver)
		Whole esters	SiO_2 fractions			Whole esters	SiO_2 fractions					
			E–H 7:93	C–M 9:1	M–C 4:1		2 C–M 9:1	3 M–C 4:1	4 M 100	5 M 100 M–C 9:1		
12:0	–	T	T	T	1.4	–	–	–	–	–	–	–
14:0	–	4.4	4.6	3.2	3.6	2.2	1.1	2.0	2.9	3.5	3.3	3.9
14:1	?	0.1	0.1	0.2	T	–	–	–	–	–	–	–
15:0	–	0.2	0.7	0.3	0.3	0.3	0.4	0.7	0.9	0.7	T	–
15:1	?	T	T	T	T	–	–	–	–	–	–	–
16:0	–	13.7	15.2	9.8	19.7	27.6	16.6	27.8	39.1	35.5	20.3	15.4
16:1	9a	9.0	9.1	9.2	6.3	0.4	0.4	0.5	1.0	1.5	6.4	5.9
16:2	9, 12	0.5	0.4	0.8	0.4	0.2	0.1	0.1	0.1	0.1	T	–
16:3	6, 9, 12	0.1	0.1	0.1	0.1	0.1	T	0.1	0.2	0.1	–	–
17:0	–	0.4	0.4	0.9	0.4	0.3	0.4	0.3	0.4	0.1	T	–
17:1	?	0.1	0.1	0.2	0.1	0.1	T	0.1	0.3	0.1	T	–
18:0	–	1.8	5.8	2.9	6.4	4.4	8.2	5.1	2.0	0.8	4.7	2.3
18:1	9a	16.3	16.2	12.6	16.1	4.9	4.8	3.8	4.0	3.6	8.1	16.4
18:2	9, 12	0.8	1.2	1.5	1.7	0.3	0.2	0.3	0.2	0.1	T	1.5
18:3	9, 12, 15	0.4	0.5	1.1	2.2	0.1	T	T	T	T	–	0.9
18:3	6, 9, 12	0.3	0.5	0.3	0.3	–	–	–	–	–	–	–
18:4	6, 9, 12, 15	0.8	0.6	2.4	0.5	0.1	T	T	T	T	0.4	0.7
19:1	?	0.1	T	T	T	T	T	T	T	T	–	–
20:1	11a	12.4	13.4	9.1	12.5	4.9	7.7	3.6	3.2	1.7	9.4	11.8
20:2	11, 14	0.3	0.2	0.3	0.3	0.3	0.2	0.2	0.2	0.3	T	–
20:3	8, 11, 14	0.1	T	T	T	–	–	–	–	–	–	–
20:4	5, 8, 11, 14	0.4	0.5	0.5	0.3	0.8	1.1	0.8	0.2	0.4	0.5	1.3
20:4	8, 11, 14, 17	0.4	0.4	1.0	0.2	T	T	T	T	T	T	0.6
20:5	5, 8, 11, 14, 17	13.9	10.6	16.6	5.3	15.8	21.2	14.6	6.8	8.5	18.9	12.1
22:1	11a	8.2	7.3	4.5	3.9	0.5	0.2	0.2	0.1	0.1	2.6	7.7
22:4	7, 10, 13, 16	0.3	0.6	0.3	0.8	0.1	T	0.2	T	0.2	0.5	–
22:5	4, 7, 10, 13, 16	0.1	T	T	T	T	T	T	T	T	T	–
22:5	7, 10, 13, 16, 19	1.3	0.6	1.2	1.9	0.3	0.2	0.5	0.2	0.2	T	1.3
22:6	4,7,10,13,16,19	16.9	12.0	22.7	16.1	37.1	36.1	40.7	39.9	43.1	24.6	15.8
24:1	?	0.3	0.4	0.2	0.2	–	–	–	–	–	0.4	2.5
Total:												
Saturated FA		20	27	17	32	34	26	35	45	41	28	22
Monounsaturated FA		44	46	35	39	11	13	8	8	7	27	42
Polyunsaturated FA		37	28	49	30	55	59	58	48	53	45	34

[a]Other isomers may be present.

The fatty acid composition was characterized by high percentages of docosahexaenoic and palmitic acids and a lower content of eicosapentaenoic and monounsaturated fatty acids than Fraction 2.

Fraction 4 was eluted with 100% methanol and Fraction 5 with 100% methanol followed by M–C 9:1. These fractions were not analyzed by TLC, but the fatty acid composition showed the following trends from Fractions 2 and 3: a high percentage of docosahexaenoic acid (22:6), decreasing amounts of stearic acid and a corresponding increase in palmitic acid, decreasing amounts of eicosapentaenoic (20:5) and eicosenoic (20:1) acids, and an increase in myristic acid (14:0).

The methyl esters of squid flesh prepared after saponification with ethanolic KOH, removal of the unsaponifiable matter and esterification of the fatty acids with boron trifluoride–methanol, presented some analytical problems. The GLC analysis indicated that the iodine value should be about 230 whereas Wijs method only gave values of about 200. Since the unsaponifiable matter had been removed, it was unlikely that inert material from that source was responsible for the discrepancy. A precipitate was noted on prolonged standing at room temperature, and the clear top layer had an iodine value that checked with GLC analysis. A TLC analysis of the whole esters, vs. a sample of cod flesh lipids, indicated the presence of two components giving spots in the phospholipid region. One spot was located in the sphingomyelin–lysolecithin area and gave ultraviolet fluorescence after spraying with 0.2% 2,7-dichlorofluorescein. The other spot was located between lecithin and phosphatidyl ethanolamine and gave no such fluorescence. Further identification of the substances was not attempted so it is difficult to say if incomplete saponification of the lipids could have caused the impurity or whether it was possibly a compound that behaved like a fatty acid on saponification, extraction, acidification, and esterification.

Viscera Lipids

Although no recognizable food could be found in the stomachs of the squid, it is possible that part of the lipid would be from this source. Since the viscera were quite watery they were not weighed and the percentage lipids was not determined. The fatty acid composition was intermediate between the composition of the liver and flesh lipids.

CONCLUSIONS

The overall fatty acid composition of the mature squid (*Illex illecebrosus* (Le Sueur)) differed from those reported for plankton-feeding molluscs and generally resembled that of the fish taken as food. A significant enrichment of the liver lipids in polyunsaturated fatty acids contributed to an iodine value markedly higher than in the depot fats of the food species such as capelin. The flesh phospholipids of the lecithin and cephalin types resembled those found in teleostean fish in fatty acid composition, but unidentified phospholipids also occurred to a significant extent.

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Lampetra richardsoni, a New Nonparasitic Species of Lamprey (Petromyzonidae) from Western North America[1]

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ABSTRACT

A new nonparasitic species of Petromyzonidae, *Lampetra richardsoni*, the western brook lamprey, from streams of British Columbia, Washington, Oregon, and possibly Alaska, is described and illustrated. This species is distinguished from *Lampetra planeri* (Bloch), the European brook lamprey (with which it has long been regarded as identical), by dentition, body proportions, pigmentation of the head and tail, and geographical distribution. The description is based on an examination of 275 specimens (69 transformed individuals and 206 ammocoetes).

INTRODUCTION

In our studies of the Petromyzonidae,[2] we have been puzzled by references to the occurrence of the European brook lamprey, *Lampetra planeri* (Bloch), along the western coast of North America, particularly since this species is absent from Asia (Jordan and Hubbs, 1925, p. 98; Berg, 1948, pp. 25, 50).

Authors who have stated that *L. planeri* occurs in western North America are: Günther (1870, p. 505, as *Petromyzon branchialis*), Creaser and Hubbs (1922, p. 13), Hubbs (1925, pp. 590, 592–597), Schultz (1929, p. 45; 1930a, pp. 5, 23, 24; 1930b, p. 137), Holly (1933, p. 31), Schultz and DeLacy (1935, p. 366), Schultz (1936, p. 130), Murphy (1941, p. 165), Hubbard (1941, p. 204), Dill (1946, p. 54), Hubbs and Wallis (1948, p. 142), Shapovalov and Dill (1950, p. 385), Eddy (1957, p. 31), Scott (1958, p. 3), Slastenenko (1958), Carl *et al.* (1959, p. 28), American Fisheries Society, Committee on Names of Fishes (1960, p. 6), Clemens and Wilby (1961, p. 68).

The absence of this species from Asia, and our recent conclusion that the European *Lampetra fluviatilis* (Linnaeus), a parasitic species, is represented in western North America by a distinct endemic parasitic species (Vladykov and Follett, 1958), have led us to suspect that the nonparasitic brook lamprey of western North America would also prove to be distinct from its European

[1]Received for publication September 8, 1964.

[2]The stem of the name of the type-genus of this family is *Petromyzon-*, not *Petromyzont-*; therefore the correct family name is Petromyzonidae, not Petromyzontidae (Gill, 1894, p. 110). Support for this conclusion appears in the rules enunciated by Miller (1897, pp. 138–139).

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congener. We now describe such a distinct form, *Lampetra richardsoni*, sp. nov. It is named in honour of Sir John Richardson, the author of several important works on fishes from North America.

METHODS

All measurements and counts were typically made on the left side of the specimen.

MEASUREMENTS

All measurements are expressed in percentages of the total length of the specimen; the disc length (d) is also expressed in percentage of the branchial length (B_1–B_7). The following abbreviations are used:

a–C *Tail length*, the distance from the posterior edge of the cloacal slit to the end of the caudal fin.

B_7–a *Trunk length*, the distance from the posterior edge of the last (7th) branchial opening to the anterior edge of the cloacal slit.

B_1–B_7 *Branchial length*, the distance from the anterior edge of the first branchial opening to the posterior edge of the last (7th) branchial opening.

d *Disc length*, longitudinal diameter, with the oral fimbriae included, measured with the disc closed.

d–B_1 *Prebranchial length*, the distance from the anterior edge of the disc (in transformed individuals) or of the upper lip (in ammocoetes) to the anterior edge of the first branchial opening.

hD_2 *Second dorsal fin maximum height*, measured along the highest fin ray.

O *Eye length*, the horizontal diameter of the eye.

TL *Total length*, the distance from the anteriormost oral fimbria to the end of the caudal fin.

Since the axial skeleton of the lampreys consists of the notochord only, a fresh specimen shrinks in length after being placed in a 4–5% formalin solution. This shrinkage (2–5 mm in specimens about 150 mm long) can be observed even after preservation of a few months, especially if the specimen has been placed in a stronger solution. Moreover, in current practice the length of lampreys is measured to the nearest millimeter only. These are the main reasons why certain irregularities in the body proportions of a sample of lampreys exist.

There is also a sexual dimorphism in body proportions.

COUNTS

TRUNK MYOMERES

Only the myomeres between the last (7th) branchial opening and the anterior edge of the cloacal slit are counted.

TEETH

A cusp distinctly divided at its tip is counted as 2. (Regardless of the species, only half-grown transformed lampreys exhibit well-developed teeth on the disc. Spawning individuals, which are much more easily caught, are usually not suitable for an accurate study of the dentition, as their teeth are often worn or may even have been lost.)

To illustrate the various types of teeth, a schematic drawing of the disc of the Pacific lamprey *Entosphenus tridentatus* (Fig. 1) has been added. The terms *transverse* (instead of *anterior*) *lingual lamina*, and *longitudinal* (instead of *posterior*) *lingual laminae*, are used in accordance with Hubbs and Trautman (1937, p. 32).

Stages of Maturity

In early stages of transformation (maturity stage 1 or 2), nonparasitic lampreys usually burrow into the bottoms of brooks or hide under stones; thus they are rarely collected. Upon completion of transformation, they gradually approach their spawning places and are more easily caught. Since they do not feed after transformation, they live only about 4–6 months. They die after spawning, but their dead bodies are infrequently seen, because they disintegrate rapidly under the stones of brooks or are eaten up by aquatic insects or fishes. Therefore, for practical purposes, the sexual maturation of nonparasitic lampreys may be confined to three stages, corresponding to the similar stages in the parasitic forms:

Stage 3. *Early maturity*: sexual products rather small; dorsal fins separated.

Stage 4. *Prespawning period*: sexual products well developed; dorsal fins adjoining.

Stage 5. *Spawning period*: sexual products occupying entire body cavity and extrusile by slight pressure on abdomen; dorsal fins contiguous; an "anal fin" well developed in females.

For further details on the stages of maturity in lampreys in general, see Vladykov and Follett (1958).

Ammocoetes

In the identification of ammocoetes the number of trunk myomeres, the body proportions, the separation of the dorsal fins, and the shape of the caudal fin are used. However, the principal differences between species consist in the pattern and intensity of the pigmentation of different sections of the head and tail.

MATERIAL EXAMINED

Our material of *L. richardsoni* consists of 69 transformed individuals (52 from British Columbia, 15 from Washington, and 2 from Oregon) and 206 ammocoetes (48 from British Columbia and 158 from Washington).

Data (if known) for each collection appear in the following sequence:

1. Tag number: (a) of the Department of Fisheries, Quebec (the letter B [= blanc], J [= jaune], R [= rouge], or V [= vert] indicates the colour [white, yellow, red, or green] of a vinylite tag); or (b) other types of vinylite tags: rY, round yellow; rW, round white; sG, small oval green; sW, small oval white; and sW with letters "VDV." (These tags are very useful in readily identifying individual specimens.)
2. Number of specimens.
3. Sex (if transformed specimen).
4. Total length.
5. Locality.
6. Date of collection.
7. Collector.
8. Name of repository museum, abbreviated as follows:
   CAS, California Academy of Sciences, San Francisco
   NMC, National Museum of Canada, Ottawa
   ROMZ, Royal Ontario Museum of Zoology
   UBC, University of British Columbia, Vancouver
   UMMZ, University of Michigan, Museum of Zoology
   USNM, United States National Museum
   UW, University of Washington, Seattle
   VM, British Columbia Provincial Museum, Victoria.
9. Catalogue number in the repository museum

HOLOTYPE

4554R (♂, 154 mm): Smith Creek, Cultus Lake, British Columbia; May 30, 1942; G. C. Carl; NMC 64-57.

PARATYPES

BRITISH COLUMBIA

4974R–4977R (4 ammocoetes, 86–104 mm), and without vinylite tags (13 ammocoetes, 35–100 mm): Millstone River, Nanaimo, Vancouver Island; March 6, 1952; E. W. Burridge.

3608V–3610V (1 ♂, 2 ♀, 91–108 mm): near Nanaimo, Vancouver Island; 1954; W. E. Ricker.

4919R, 4928R–4952R, and 1 specimen without vinylite tag (19 ♂, 8 ♀, 120–148 mm), and without vinylite tags (31 ammocoetes, 20–72 mm): Holmes Creek, near Duncan, Vancouver Island; May 7, 1950; P. Parizeau.

3835V–3836V (1 ♂, 120 mm; 1 ♀, 130 mm), and 4637R–4640R (1 ♂, 3 ♀, 101–139 mm): Holmes Creek, near Duncan, Vancouver Island; May 8, 1939; G. C. Carl; UMMZ 126336.

3929V (1 ♂, 153 mm): Smith's Fall Stream, Cultus Lake; May 5, 1923; ROMZ 577.

4444R–4448R, 4550R–4553R, 4555R–4559R and 1 specimen without vinylite tag (9 ♂, 6 ♀, 131–154 mm): Smith's Creek, Cultus Lake; May 30, 1942; G. C. Carl; VM 569.

WASHINGTON

Without vinylite tag (1 young transformed, 133 mm): Washington Creek, ½ mile east of Chimacum; UW 967.

3470V–3479V (10 ammocoetes, 95–128 mm): Ames Lake; August 16, 1951; Roy Banner.

3500V–3516V (1 ♂, 119 mm, 1 ♀, 114 mm, and 15 ammocoetes, 102–137 mm) and without vinylite tags (83 ammocoetes, 20–115 mm): North Creek, near Alderwood, 15 miles north of Seattle; January 16, 1955; A. D. Welander.

FIG. 1–9

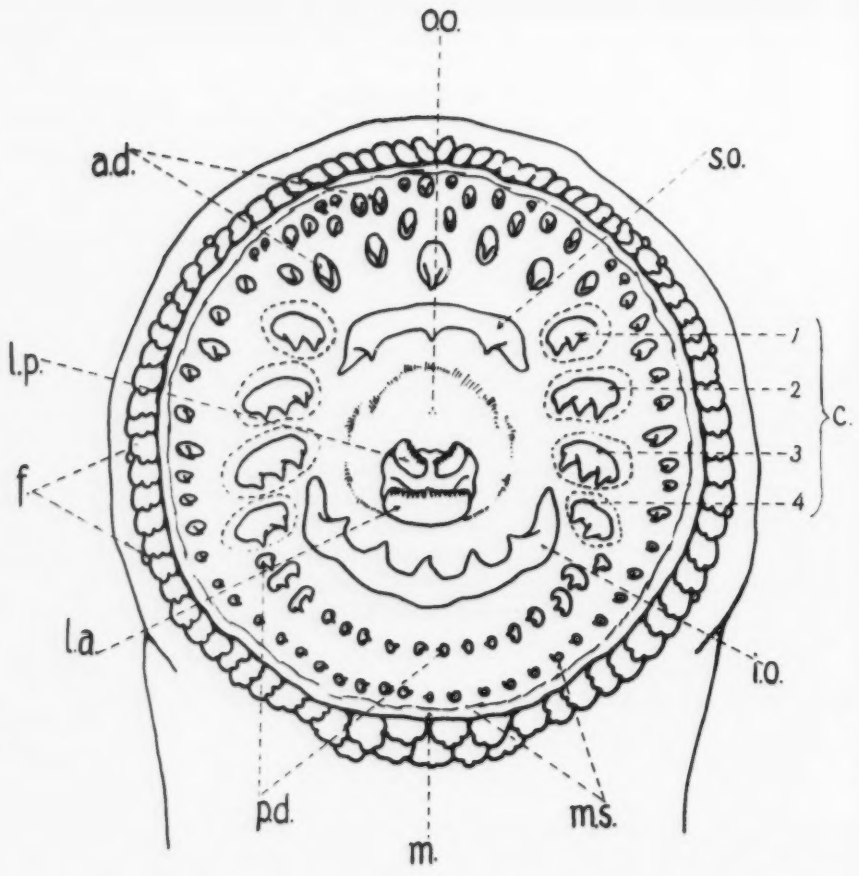

FIG. 1. Schematic drawing of the disc and dentition in the Pacific lamprey, *Entosphenus tridentatus*. The significance of the abbreviations used in this drawing is as follows:

a.d. — *maxillary labials* or rows of cusps of the anterior field
c. — four *circumorals* or enlarged laterals
f. — *oral fimbriae* or leathery marginal appendages
i.o. — *infraoral lamina* or infraoral cusps
l.a. — *transverse*, or *anterior, lingual lamina*
l.p. — *longitudinal*, or *posterior, lingual laminae*
m. — *marginal membrane*
m.s. — *marginal series* or *marginals*
o.o. — *oesophageal opening*
p.d. — *mandibular labials* or infraorals, a row (rows) of cusps of the posterior field
s.o. — *supraoral lamina* with its three cusps

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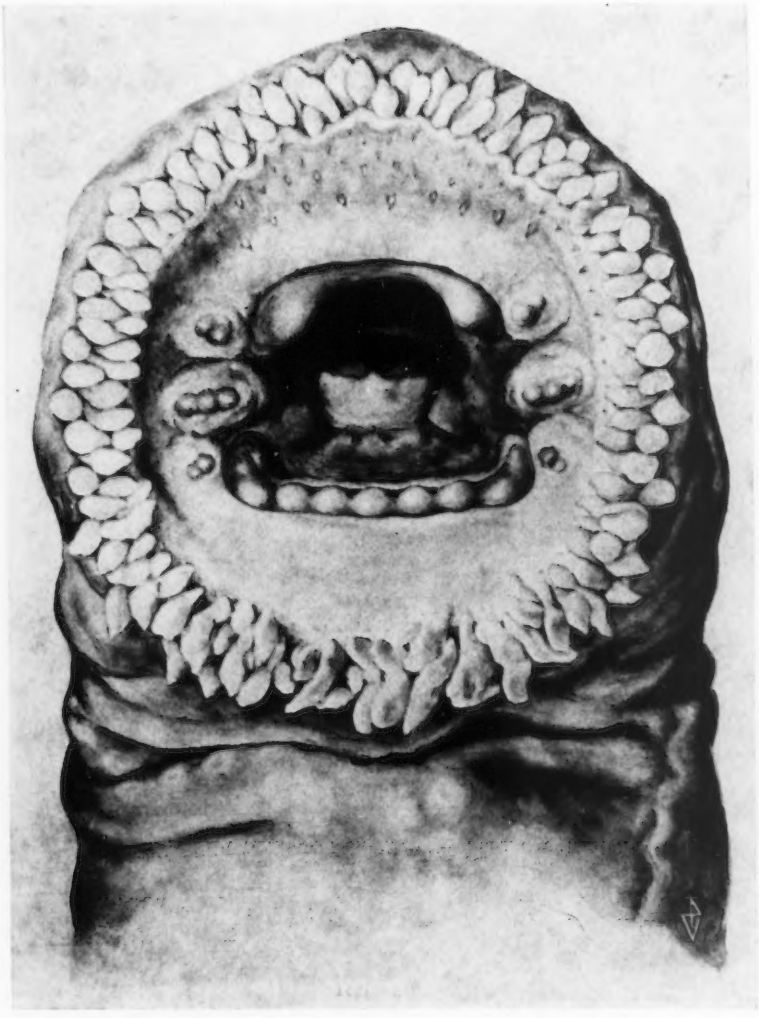

FIG. 2. Disc of a specimen of *Lampetra planeri* (Tag 1435J), spawning male, TL 120 mm, River Allier (Culhat), France.

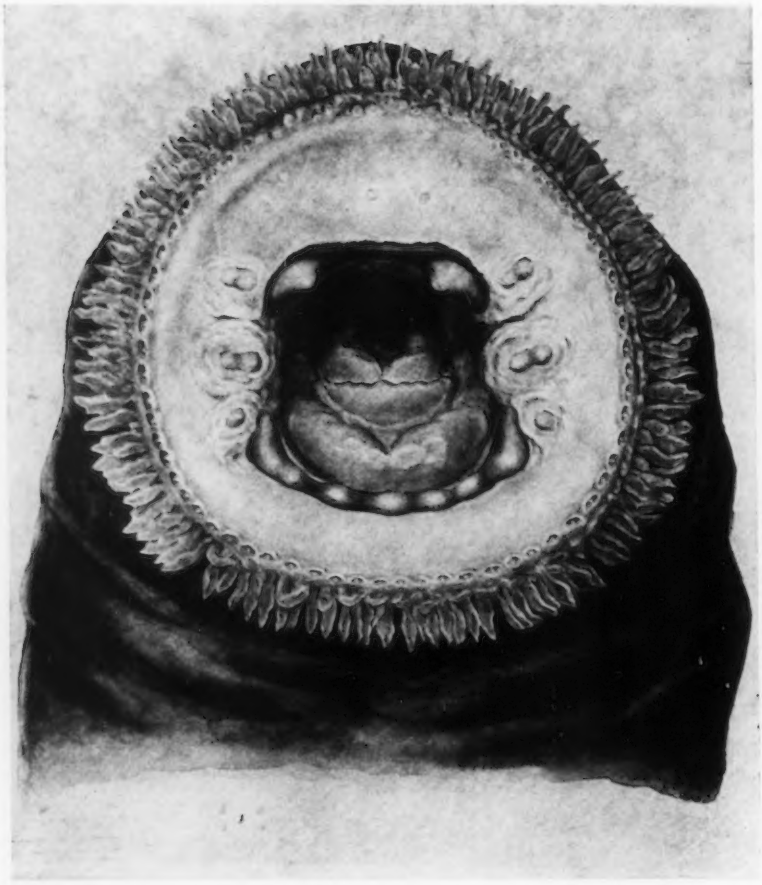

FIG. 3. Disc of the holotype of *Lampetra richardsoni* (Tag 4554R), prespawning male, TL 154 mm, Smith Creek, Cultus Lake, British Columbia.

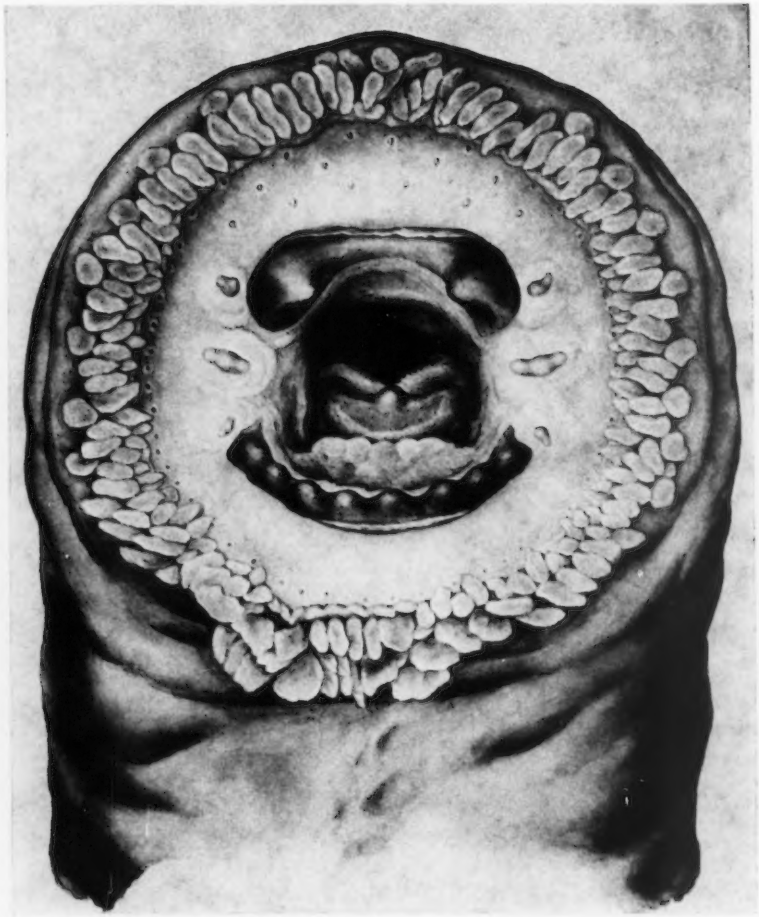

FIG. 4. Disc of the paratype of *Lampetra richardsoni* (Tag 4445R), prespawning male, TL 152 mm, Smith Creek, Cultus Lake, British Columbia. This specimen, selected for illustration at the beginning of the study, differs from the holotype (Fig. 3) only in possessing tricuspid middle circumorals, instead of the usual bicuspid circumorals.

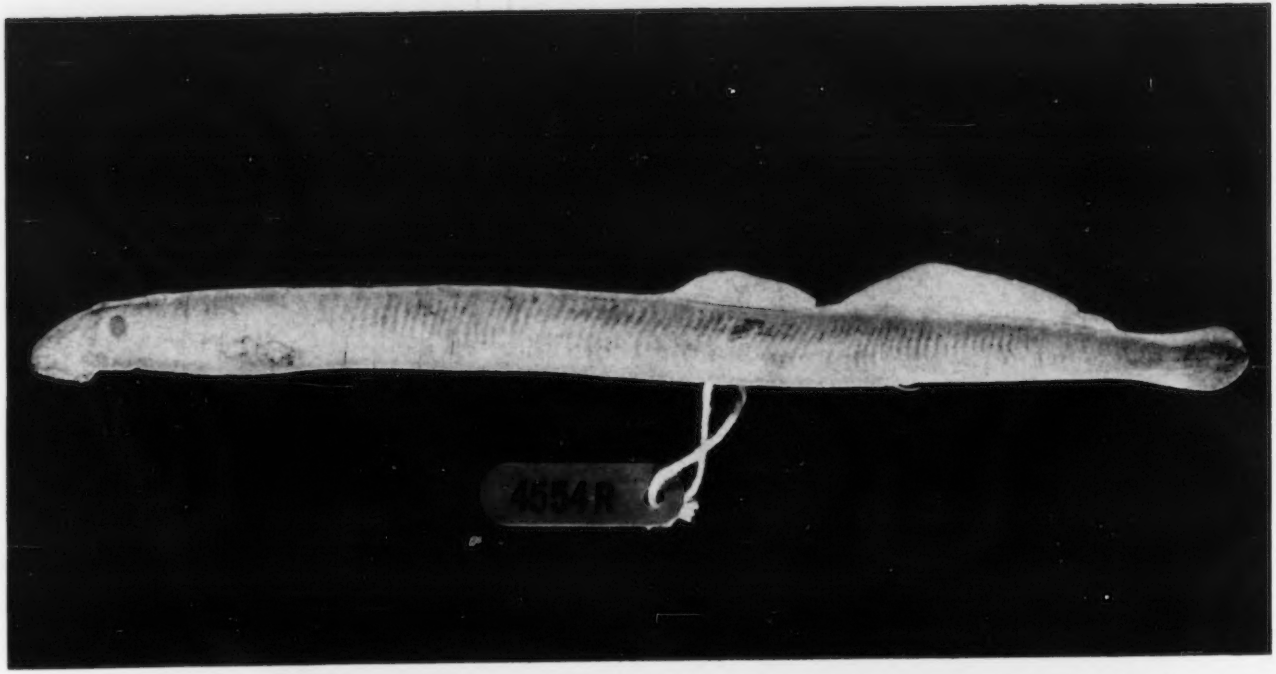

FIG. 5. Holotype of *Lampetra richardsoni* (Tag 4554R), prespawning male, TL 154 mm, Smith Creek, Cultus Lake, B. C.

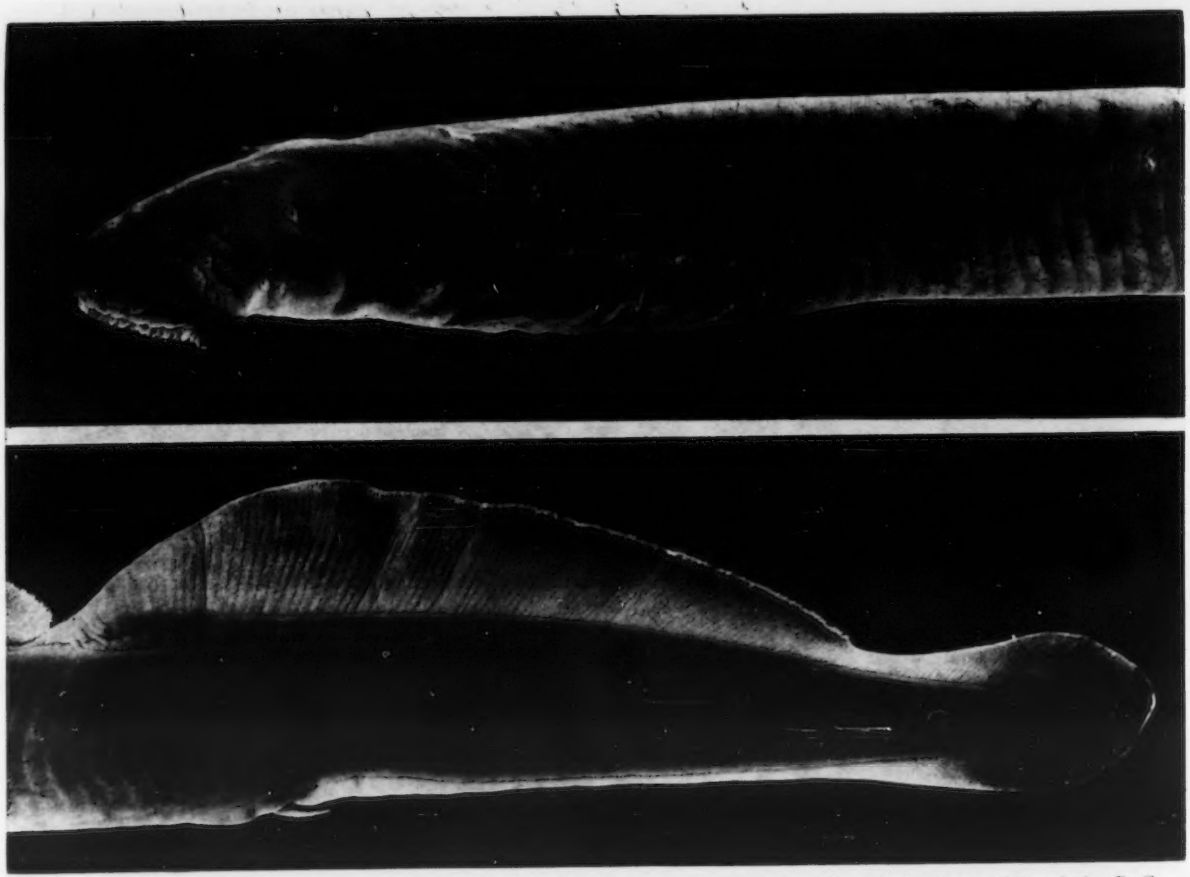

FIG. 6. Head and tail regions of a prespawning male (Tag 4556R) of *Lampetra richardsoni* from Smith Creek, Cultus Lake, B. C.

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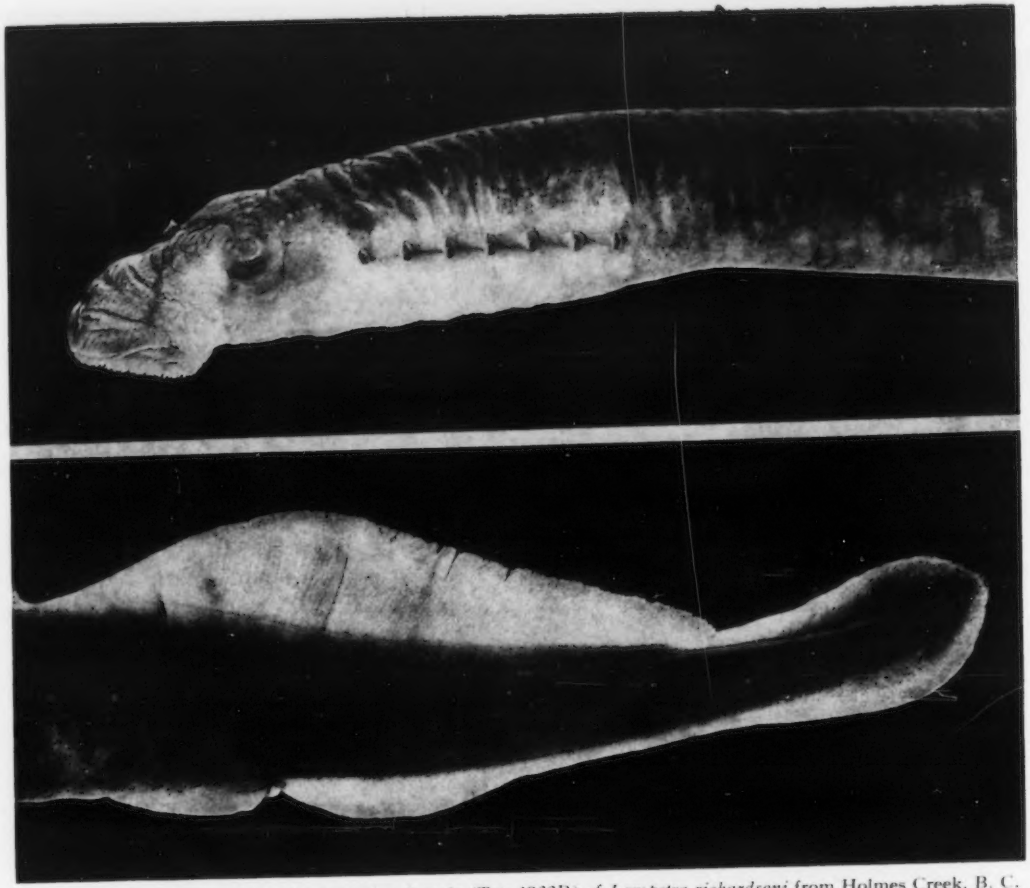

FIG. 7. Head and tail regions of a spawning female (Tag 4932R) of *Lampetra richardsoni* from Holmes Creek, B. C.

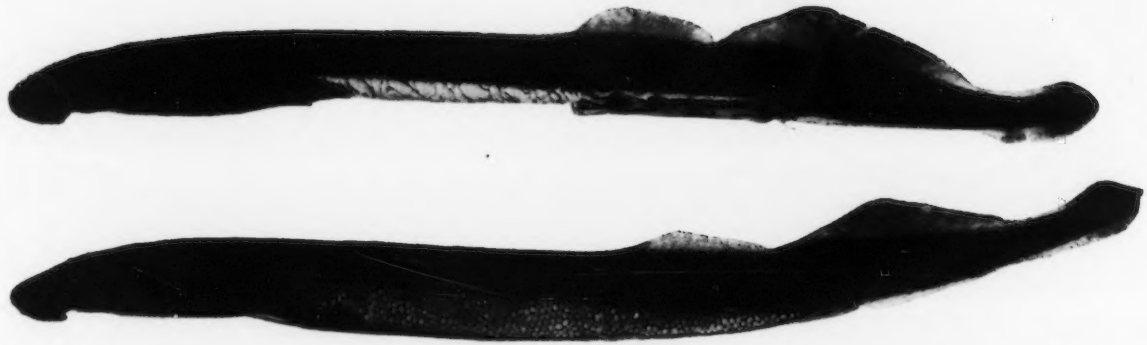

FIG. 8. Comparison between gonads in prespawning male (Tag 4557R) and prespawning female (Tag 4550R) of *Lampetra richardsoni* from Smith Creek, Cultus Lake, B. C. In addition to differences in aspect of gonads, note the larger disc, higher second dorsal fin, and long genital papilla in male. Female has an extended ventral fold ("fin") and an upturned tail.

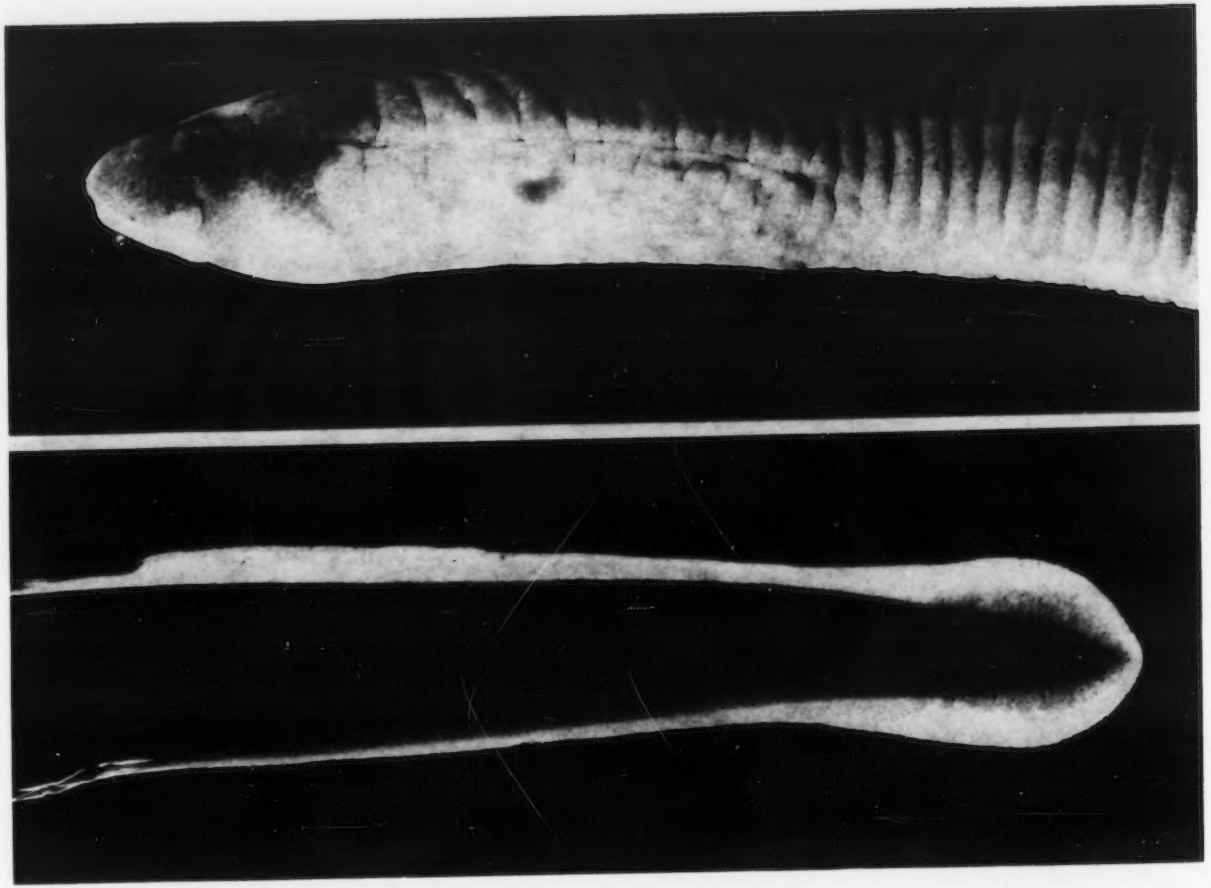

FIG. 9. Head and tail regions of an ammocoete (Tag 3507V) of *Lampetra richardsoni*, TL 118 mm, from North Creek, Washington.

Without vinylite tags (6 ammocoetes, 46–95 mm; February 26, 1929; UW 355); without vinylite tags (9 ammocoetes, 62–107 mm; UW 245); 3986V–3987V (2 young transformed, 112–114 mm; March 26, 1929; UW 245); without vinylite tags (4 ♂, 2 ♀, 93–110 mm, and 3 ammocoetes, 60–82 mm; July 4, 1929; UW 359); 4970R–4973R (4 ammocoetes, 86–130 mm); and without vinylite tags (5 ammocoetes, 67–116 mm; February 18, 1940; UW 4750): all from Evans Creek, Lake Washington drainage, east of Redmond; collected by students of the School of Fisheries, University of Washington.

3837V–3838V (2 ♂, 80–141 mm): Matthews Creek, near Seattle; April 1931; L. P. Schultz; UMMZ 97216.

Without vinylite tag (1 ammocoete, 126 mm): Puget Sound, on sandspit south of Ballard Locks, Seattle; May 23, 1939; Foster and class; UW 4668.

3570V–3577V (8 ammocoetes, 86–175 mm): Steel Lake; May 9, 1940; A. D. Welander.

Without vinylite tags (6 ammocoetes, 55–70 mm): Clover Creek, 2 miles south of Tacoma, Pierce Co.; February 20, 1936; H. J. Leraas; UMMZ 126160.

Without vinylite tag (1 ♂, 135 mm): Yakima River, ¼ mile below Easton Dam; July 1950; UW 6233.

Without vinylite tags (5 ammocoetes, 29–99 mm): Chehalis River, creek west of Satsop; December 30, 1929; L. P. Schultz; UW 547.

2190rY–2192rY (1 ♂, 107 mm, and 2 ammocoetes, 119–120 mm): Chehalis River; May 23, 1957; H. Evans.

2187rY (1 ammocoete, 149 mm): Yakima River, Ellensburg; June 18, 1958; H. Evans.

OREGON

Without vinylite tag (1 transformed): creek tributary to Umpqua River near Reedsport; May 26, 1930; L. P. Schultz and students of University of Washington.

Without vinylite tag (1 transformed): Willamette River, Columbia system; August 1929.

One or more paratypes are being deposited in museums listed on page 142.

DESCRIPTION OF *LAMPETRA RICHARDSONI*, SP. NOV.

WESTERN BROOK LAMPREY

Lampetra planeri of authors (western North America, in part); not *Petromyzon planeri* Bloch, 1784, p. 47.

Figures 3–9

DIAGNOSIS

This nonparasitic species of *Lampetra* differs from *L. planeri* (Bloch) (Fig. 2) in having (1) the middle circumoral predominantly bicuspid (instead of tricuspid), (2) the branchial region longer, (3) the head regions of ammocoetes, and the caudal fin of ammocoetes and transformed individuals, with (instead of without) pronounced dark pigmentation, and (4) a geographical distribution restricted to western North America instead of Europe.

In the present publication, *Lampetra richardsoni* will be compared only with *L. planeri*. The information on *L. planeri* is taken from Vladykov (1955), unless some other reference is noted.

HOLOTYPE

The holotype, a male 154 mm in total length (when received), is referable to maturity stage 4 (prespawning period). Its proportional measurements, expressed in percentages of the total length, are as follows: prebranchial length, 11.7; branchial length, 10.4; trunk length, 46.1; tail length, 29.2; maximum

FIG. 9. Head and tail regions of an ammocoete (Tag 3837V) of *Lampetra richardsoni*. Vladykov and Follett, J. Fish. Res. Bd. Canada

height of second dorsal fin, 5.9; disc length, 5.9; eye length, 2.6. The disc length in percentage of the branchial length is 56.3. There are 64 trunk myomeres.

The teeth comprise about 15 maxillary labials; 3 circumorals on each side, all bicuspid except 1 (the third on the right is unicuspid); the transverse lingual lamina with 4 minute cusps on each side of the enlarged median cusp; the infraoral lamina with 7 cusps (Fig. 3).

Colour in 4–5% formalin is greyish, darker above, with an area of dark pigment discernible on the caudal fin.

Transformed Specimens

TOTAL LENGTH

The total lengths of transformed specimens studied are given in Tables I–IV; the average lengths are summarized in Table V. The lengths of 16 females range from 101 to 149 mm (average 133 mm). The lengths of 29 males range from 121 to 154 mm (average 137 mm). Our specimens of *L. richardsoni* are somewhat larger than the specimens of *L. planeri* studied by Vladykov (1955, p. 218) — of which, 11 females averaged 119 mm, and 5 males, 117.2 mm. The smallest transformed specimens of *richardsoni* are a male (UMMZ 97216) 80 mm long and a female (UW 359) 93 mm long.

BODY PROPORTIONS

Detailed information on the body proportions of *L. richardsoni* is given in Tables I–IV. The summary of these proportions is presented in Table V. The greatest morphometric difference between *L. richardsoni* and *L. planeri* lies in the length of the branchial region. *L. richardsoni* has a much longer branchial region, the average length of which is 9.6 for females and 9.8 for males. For *L. planeri* the same measurements are, respectively, 8.7 and 8.6. The average length of the disc expressed as a percentage of the total length is nearly the same in the two species: in *planeri*, 5.5 for females, and 6.8 for males; in *richardsoni*, 5.6 for females, and 6.5 for males. The average length of the disc expressed as a percentage of the length of the branchial region is greater in *planeri* than in *richardsoni:* 64.5 for females, and 78.9 for males in *planeri;* 58.1 for females, and 66.7 for males in *richardsoni*. The other body proportions are quite similar; the differences can be explained by the inequality of the sizes of the specimens or the stages of maturity.

TRUNK MYOMERES

The number of trunk myomeres is nearly the same in the two species: 60–67 (average 63.2) in *richardsoni* (58 transformed specimens; Table VI); 60–65 (average 62.2) in *planeri* (53 transformed specimens; Vladykov, 1955, p. 219).

DENTITION

The general pattern of dentition of *richardsoni* is similar to that of *planeri*, but the teeth (or cusps) are less strongly developed in *richardsoni* (Fig. 3–4).

In both species, on each side of the disc are 3 enlarged lateral teeth, or circumorals, the middle one typically bicuspid in *richardsoni*, and tricuspid in *planeri*. Although the middle circumoral is sometimes tricuspid in *richardsoni*, it is much more often bicuspid: among 23 specimens (Table VII), 21 (91%) have the middle circumoral bicuspid on one or both sides of the disc (bicuspid on both sides in 19 specimens, on one side in 2 specimens).

Among 47 specimens of *planeri*, Zanandrea (1958, p. 211) found 40 (85%) with the middle circumoral tricuspid on one or both sides of the disc (tricuspid on both sides in 37 specimens, on one side in 3 specimens).

The supraoral lamina, which lacks a median cusp, is broad, with a blunt cusp at each end, in both *richardsoni* and *planeri*.

The average number of infraoral cusps is somewhat different in the two species: in *richardsoni*, 7.7 (7.8 in 43 specimens from British Columbia, 7.4 in 11 from Washington; Table VIII); in *planeri*, 7.1 (Vladykov, 1955, p. 218). To determine whether this difference between the two species is significant, further study is required.

The cusps on the transverse lingual lamina are very small in *richardsoni*; these cusps are more sharply defined in *planeri*.

The cusps of the longitudinal lingual laminae are small, rather irregular, and practically uncountable, in both species.

The maxillary labials are better developed in *planeri* than in *richardsoni*.

The mandibular labials are absent in both *richardsoni* and *planeri*, as, typically, in all species of *Lampetra*. The presence of "mandibular labials" in several specimens of *L. zanandreai* was noted by Zanandrea (1958, pp. 211–213). He stated that these minute "mandibular labials" ("piccoli denti nel labbro inferiore") were especially noted in live specimens ("quasi sempre in animali vivi, esaminati in stato di narcosi; ottenuta con una soluzione di metiluretano"). To reach a definite conclusion on the nature of such "mandibular labials", further study is required.

COLOURATION

Our specimens of both *richardsoni* and *planeri* fixed in 4–5% formalin have the same general colouration. The sides and back are greyish and the lower surface is whitish.

The principal difference between the two species lies in the extent of pigmentation of the caudal fin. In transformed individuals, as well as in ammocoetes, there is a conspicuous area of dark pigment on the caudal fin of *richardsoni* (Fig. 5–6–8). The pattern in transforming individuals is more similar to that of adults than to that of ammocoetes. In *planeri*, the caudal fin remains unpigmented throughout life, except in spawning individuals, in which a weak "peppered" pigmentation could be discerned. This "spawning pigmentation" of *planeri* is, however, much less developed than that described for *L. fluviatilis* by Vladykov and Follett (1958). The dark pigmentation brings *richardsoni* closer to *L. zanandreai* (Vladykov, 1955) than to *planeri*. (This similarity between *richardsoni* and *zanandreai* is rather superficial, since *zanandreai* has a much smaller number of trunk myomeres and differs in other respects as well.)

SEXUAL DIMORPHISM

The external appearance of spawning males and females of *richardsoni* is similar to that of other species of *Lampetra* as described by Zanandrea (1940), Vladykov (1955), Vladykov and Follett (1958), and others (Fig. 6–8).

Sexual differences in body proportions of *richardsoni* are given in Tables I–V. The males have a greater disc length (d), prebranchial length ($d–B_1$), tail length (a–C), and second-dorsal-fin height (hD_2); the females have a greater trunk length ($B_7–a$).

AMMOCOETES

TOTAL LENGTH

The total lengths of the 206 ammocoetes of *L. richardsoni* studied range from 20 to 175 mm. The average length of the larger ammocoetes is 113 mm (Table XV). The average lengths of ammocoetes of *L. planeri* from the Morge and Auzon Rivers in France were 145 mm and 149 mm, respectively (Vladykov, 1955, p. 221).

BODY PROPORTIONS

Both very young and large ammocoetes of *L. richardsoni* were studied (Tables IX–XV). There are definite changes in body proportions of young and large individuals (Table XI). The prebranchial length ($d–B_1$) and the tail region (a–C) are shorter in younger ammocoetes than in older ammocoetes (Tables XI and XV). A comparison of body proportions of ammocoetes of different species should therefore be made within corresponding length-groups.

Body proportions of ammocoetes of *richardsoni* and *planeri* of comparable size are quite similar. However, the branchial length of *richardsoni* is somewhat greater (12.5%) than that in two samples of *planeri* (11.1% and 10.6%). It is possible that this difference is due to some extent to inequality in sizes of ammocoetes studied, although transformed specimens of *richardsoni* also have a greater branchial length.

TRUNK MYOMERES

The number of trunk myomeres in 121 ammocoetes of *richardsoni* (Table VI) ranges from 57 to 65 (average 60.7)—the same number as in ammocoetes of *planeri* (Vladykov, 1955, p. 219).

PIGMENTATION

The main distinction between ammocoetes of *richardsoni* (Fig. 9) and *planeri* consists in the pattern and extent of dark pigmentation on the tail and certain parts of the head. (See Table XVI for variation in the degree of pigmentation in *richardsoni*.)

CAUDAL FIN. The pigmentation of the caudal fin is a very important character. The pattern distinctive of a species is already present in young ammocoetes; only the intensity and extent of this pigmentation become somewhat more pronounced in the larger individuals. All ammocoetes of *richardsoni*,

upon reaching a total length of about 60 mm, develop dark pigmentation similar to that of the larger ammocoetes. Among 64 ammocoetes of *planeri*, only 1 (1.6%) had pigmentation on the tail around the notochord (Vladykov, 1955, p. 221).

HEAD REGION. In the head region, there are four principal areas of taxonomic significance (see Vladykov, 1950, p. 80, fig. 4):

1. *Upper lip.* The pattern of pigmentation of the hoodlike upper lip is characteristic of the species. The upper part of the upper lip is well pigmented in all species, but the lower part exhibits a variety of conditions. A definite pigmentation of the lower part of the upper lip was observed in every ammocoete of *richardsoni*, but none was discernible in any ammocoete of *planeri*.

2. *Subocular region.* The subocular region is situated in front of the first branchial opening, immediately below the eye. (The eye is usually discernible in very young and old ammocoetes. In the intermediate sizes (60–100 mm), there is a darkish spot on the skin or a slight depression which corresponds to the position of the eye.) Most ammocoetes (87.3%) of *richardsoni* show strong pigmentation of the subocular region; ammocoetes of *planeri* lack subocular pigmentation (see Vladykov, 1955, plate 2, fig. b).

3. *Prebranchial region.* The prebranchial region is situated behind the eye and below the first branchial opening. The prebranchial blotch is always present in ammocoetes of *richardsoni* irrespective of their size; this blotch is moderate or strong in 91.5% of our larger ammocoetes. It was present in only 28% of the ammocoetes of *planeri* studied by Vladykov (1955, p. 220).

4. *Branchial region.* The only part of the branchial region of taxonomic importance is the upper section, above the horizontal groove that unites the branchial openings. This upper section is well pigmented in 91.5% of our larger ammocoetes of *richardsoni*; it was pigmented (weakly) in fewer than 5% of ammocoetes of *planeri* (Vladykov, 1955, p. 221).

The lower section of the branchial region is unpigmented in most species, including *planeri*. In the larger ammocoetes of *richardsoni*, there are irregular pigmented blotches on the lower surface of the branchial region and of the body. This section is similarly pigmented in larger ammocoetes of *zanandreai* (Vladykov, 1955).

GEOGRAPHICAL DISTRIBUTION

Geographical distribution—as well as morphology and pigmentation—distinguishes *richardsoni* from *planeri*.

Lampetra planeri is limited to freshwater streams of the Atlantic drainage of Europe (Vladykov, 1955 and 1958; Zanandrea, 1958). *Lampetra richardsoni* is found in freshwater streams of the Pacific drainage of North America. So far as our material permits us to judge, *richardsoni* inhabits waters of British Columbia, Washington, and Oregon.

It is possible that *richardsoni* may be found in Alaska as well. In the early period of the present studies, one of the authors (V. D. V.) examined and identified as *L. richardsoni* two ammocoetes (USNM 84411 and 84447), 106

and 175 mm in length, from Lake McDonald, Alaska, collected by Hauser on May 8 and June 5, 1907.

The geographical distribution of *L. richardsoni* and *L. planeri*, and their relationship to each other, are similar to those of a pair of parasitic species, *L. ayresii* and *L. fluviatilis*, discussed by Vladykov and Follett (1958).

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TABLES I–XVI follow.

TABLE I. Body proportions (in percentages) of spawning females of *Lampetra richardsoni* from Holmes Creek, B.C., collected on May 8, 1939, and May 7, 1950.

Tag No.	Total length (TL) (*mm*)	$\frac{d-B_1}{TL}$	$\frac{B_1-B_7}{TL}$	$\frac{B_7-a}{TL}$	$\frac{a-C}{TL}$	$\frac{hD_2}{TL}$	$\frac{O}{TL}$	$\frac{d}{TL}$	$\frac{d}{B_1-B_7}$
4638R[a]	101	10.9	9.4	49.0	28.7	5.0	2.5	5.4	57.9
4637R[a]	118	11.0	9.3	50.0	28.0	5.9	2.1	5.5	59.1
4931R	120	12.5	10.8	48.3	26.7	5.8	2.5	6.7	61.5
4934R	122	11.9	9.4	51.6	26.6	5.3	2.5	6.6	69.6
4933R	123	12.3	10.2	50.8	25.2	4.9	2.4	5.8	56.0
4919R	132	12.1	9.9	46.2	26.5	5.3	2.3	6.1	61.5
4930R	134	12.3	10.5	49.3	25.8	5.2	2.2	5.6	53.6
4929R	137	11.7	9.9	49.3	27.0	5.1	2.2	5.8	59.3
4639R[a]	139	11.5	9.4	50.4	27.3	5.8	2.2	5.0	53.8
4928R	142	12.0	10.2	48.9	27.8	4.9	2.1	6.0	58.6
4932R	148	11.8	9.5	52.4	25.7	4.7	2.0	5.7	60.7
Mean	128.7	11.8	9.9	49.7	26.8	5.3	2.3	5.8	59.2

[a]Spent females collected on May 8, 1939.

TABLE II. Body proportions (in percentages) of spawning males of *Lampetra richardsoni* from Holmes Creek, B.C., collected on May 7, 1950.

Tag No.	TL (*mm*)	$\frac{d-B_1}{TL}$	$\frac{B_1-B_7}{TL}$	$\frac{B_7-a}{TL}$	$\frac{a-C}{TL}$	$\frac{hD_2}{TL}$	$\frac{O}{TL}$	$\frac{d}{TL}$	$\frac{d}{B_1-B_7}$
4937R	121	13.2	8.3	47.1	29.3	5.8	2.5	6.6	80.0
4938R	123	13.8	9.4	45.5	28.5	5.7	2.4	6.9	73.9
4939R	123	13.4	9.4	47.6	27.7	5.7	2.4	7.3	78.3
4941R	126	11.9	9.5	48.4	28.6	6.4	2.4	6.7	70.8
4942R	126	13.1	9.5	48.8	27.8	6.0	2.4	7.1	75.0
4943R	127	13.4	9.8	45.3	29.9	5.5	2.4	7.9	80.0
4944R	128	13.3	9.8	45.7	28.9	6.3	2.7	6.3	64.0
4946R	132	14.0	9.9	44.7	29.5	5.3	2.3	7.2	73.1
4947R	133	13.5	9.8	45.1	29.3	5.6	2.3	7.1	73.1
4950R	133	13.2	9.8	47.7	28.2	6.0	2.3	6.8	69.2
4936R	134	13.1	9.3	50.0	26.9	6.0	2.2	6.7	72.0
4951R	135	12.6	9.6	45.9	29.6	6.7	2.2	6.7	69.2
4948R	135	13.3	10.7	47.4	26.7	5.9	2.2	7.0	65.5
4949R	136	13.2	10.3	47.1	28.0	5.9	2.2	7.4	71.4
4935R	137	13.1	10.2	47.5	28.5	5.8	2.2	6.9	67.9
4937R	137	12.4	9.5	49.3	27.4	5.8	2.2	6.6	69.2
4952R	138	12.7	10.5	47.1	28.3	5.8	2.2	6.5	62.1
4940R	141	13.1	9.2	46.8	28.0	5.7	2.1	7.1	76.9
Mean	131.4	13.1	9.7	47.1	28.4	5.9	2.3	6.9	71.8

TABLE III. Body proportions (in percentages) of prespawning females of *Lampetra richardsoni* from Smith Creek, Cultus Lake, B.C., collected on May 30, 1942.

Tag No.	TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL	hD_2 / TL	O / TL	d / TL	d / B_1–B_7
4551R	133	11.3	9.8	51.1	26.3	5.6	2.3	5.6	57.7
4444R	144	10.4	8.7	51.7	27.1	4.9	2.1	4.9	56.0
4448R	145	10.4	9.7	53.1	25.2	4.1	2.1	4.1	42.9
4550R	146	11.3	8.6	52.1	26.7	4.8	2.4	5.1	60.0
4447R	149	11.1	8.7	52.7	26.2	4.7	2.3	5.4	61.5
Mean	143.4	10.9	9.1	52.1	26.3	4.8	2.2	5.0	55.6

TABLE IV. Body proportions (in percentages) of prespawning males of *Lampetra richardsoni* from Smith Creek, Cultus Lake, B.C., collected on May 30, 1942. Specimen No. 4554R is the holotype.

Tag No.	TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL	hD_2 / TL	O / TL	d / TL	d / B_1–B_7
4558R	131	11.8	10.3	48.1	28.3	5.3	2.3	5.7	55.6
4446R	134	12.0	9.7	50.8	26.1	4.5	2.6	6.0	61.5
4552R	142	12.3	9.9	49.6	26.8	5.6	2.5	5.6	57.1
4559R	144	11.8	10.4	47.6	28.8	5.2	2.4	5.9	56.7
4555R	147	12.3	9.5	49.0	27.9	5.1	2.7	6.1	64.3
4445R	152	10.7	10.0	52.0	29.2	4.3	2.4	5.3	53.5
4553R	153	11.8	9.5	47.1	30.4	4.9	2.6	5.6	58.6
4557R	153	12.4	9.5	49.0	27.5	5.2	2.6	6.5	69.0
4556R	153	11.4	9.2	50.0	28.1	5.2	2.3	5.2	57.1
4554R	154	11.7	10.4	46.1	29.2	5.9	2.6	5.9	56.3
–	154	11.7	10.4	48.4	27.6	4.5	2.6	5.5	53.1
Mean	147.0	11.8	9.9	48.9	28.2	5.1	2.5	5.8	58.4

TABLE V. Average body proportions (in percentages) of transformed specimens of *Lampetra richardsoni* from two British Columbia localities (from Tables I–IV).

Locality	Sex	Stage of maturity	Number of specimens	TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL	hD_2 / TL	O / TL	d / TL	d / B_1–B_7
Cultus Lake	♀	4	5	143.4	10.9	9.1	52.1	26.3	4.8	2.2	5.9	55.6
Holmes Creek	♀	5	11	128.7	11.8	9.9	49.7	26.8	5.3	2.3	5.8	59.2
Mean			16	133.3	11.5	9.6	50.4	26.7	5.1	2.3	5.6	58.1
Cultus Lake	♂	4	11	147.0	11.8	9.9	48.9	28.2	5.1	2.5	5.8	58.4
Holmes Creek	♂	5	18	131.4	13.1	9.7	47.1	28.4	5.9	2.3	6.9	71.8
Mean			29	137.4	12.6	9.8	47.7	28.3	5.6	2.4	6.5	66.7

TABLE VI. Number of trunk myomeres of *Lampetra richardsoni* from British Columbia and Washington. (a = ammocoetes; t = transformed individuals.)

Locality	Date	TL (*mm*)	Stage	Number of specimens	Number of myomeres											Mean
					57	58	59	60	61	62	63	64	65	66	67	
Cultus Lake	May 30, 1942	131–154	t	16	–	–	–	2	2	1	3	4	2	–	2	63.3
Nanaimo (Millstone River)	May 6, 1952	35–104	a	17	–	–	–	1	5	3	6	1	1	–	–	61.8
Holmes Creek	May 8, 1939	118–139	t	3	–	–	–	–	1	–	1	–	1	–	–	63.0
Holmes Creek	May 7, 1950	120–148	t	27	–	–	–	–	–	6	6	5	6	4	–	63.9
Holmes Creek	July 29, 1950	20–72	a	31	4	3	7	3	6	4	4	–	–	–	–	59.9
Total:		20–104	a	48	4	3	7	4	11	7	10	1	1	–	–	60.8
		118–154	t	46	–	–	–	2	3	7	10	9	8	4	2	63.6
Washington Creek	May 29, 1930	133	t	1	–	–	–	–	–	–	–	–	–	1	–	66.0
North Creek	Jan. 16, 1955	102–137	a	16	–	–	3	5	3	5	–	–	–	–	–	60.6
Evans Creek	Feb.–July, 1929	46–107	a	18	–	–	–	3	6	4	3	2	–	–	–	61.4
Evans Creek	Feb.–July, 1929	93–114	t	8	–	–	–	2	1	1	4	–	–	–	–	62.9
Evans Creek	Feb. 18, 1940	67–130	a	9	–	–	–	3	2	3	1	–	–	–	–	61.2
Matthews Creek	April, 1931	80–141	t	2	–	–	–	–	–	1	1	–	–	–	–	62.5
Puget Sound	May 23, 1939	126	a	1	–	–	–	–	–	–	1	–	–	–	–	63.0
Clover Creek	Feb. 20, 1936	55–70	a	5	2	1	1	–	1	–	–	–	–	–	–	58.4
Yakima River	July, 1950	135	t	1	–	–	–	–	–	–	1	–	–	–	–	63.0
Chehalis River	Dec. 30, 1929	29–99	a	6	3	1	2	–	–	–	–	–	–	–	–	57.8
Steel Lake	May 9, 1940	86–175	a	8	3	–	2	1	2	–	–	–	–	–	–	58.9
Ames Lake	Aug. 16, 1951	95–128	a	10	–	–	–	–	2	4	2	2	–	–	–	62.4
Total:		29–175	a	73	8	2	8	12	16	16	7	4	–	–	–	60.7
		80–141	t	12	–	–	–	2	1	2	6	–	–	1	–	62.4
British Columbia		20–104	a	48	4	3	7	4	11	7	10	1	1	–	–	60.8
		118–154	t	46	–	–	–	2	3	7	10	9	9	4	2	63.6
Washington		29–175	a	73	8	2	8	12	16	16	7	4	–	–	–	60.7
		80–141	t	12	–	–	–	2	1	2	6	–	–	1	–	62.4
Total:		20–175	a	121	12	5	15	16	27	23	17	5	1	–	–	60.73
		80–154	t	58	–	–	–	4	4	9	16	9	9	5	2	63.19

TABLE VII. Number of cusps on circumorals in *Lampetra richardsoni* from British Columbia. Left figure indicates number of cusps on anterior circumoral; middle figure, on middle circumoral; right figure, on posterior circumoral.

Tag No.	Sex	Side of disc	
		Right	Left
4445R	M	2–3–2	2–3–2
4448R	M	2–2–?	2–2–1
4550R	F	2–2–1	2–2–1
4551R	F	1–2–1	1–2–1
4553R	M	2–2–2	2–2–2
4554R	M	2–2–1	2–2–2
4556R	M	1–2–1	1–2–1
4557R	M	1–2–1	1–2–1
4930R	F	2–2–1	1–2–1
4933R	F	2–2–1	2–2–1
4934R	F	2–2–2	2–3–2
4936R	M	2–3–1	2–3–2
4937R	M	1–2–1	1–2–1
4938R	M	1–2–1	1–2–1
4941R	M	?–2–?	?–2–?
4942R	M	2–2–1	2–2–1
4943R	M	2–2–2	2–2–2
4944R	M	2–2–2	2–2–1
4946R	M	2–2–1	2–2–1
4948R	M	1–2–1	1–2–1
4950R	M	1–2–1	1–2–1
4952R	M	2–3–2	2–2–2
4953R	M	2–2–2	2–2–2

TABLE VIII. Number of cusps on infraoral lamina of *Lampetra richardsoni*.

Locality	Date	TL (*mm*)	Number of specimens	Number of cusps				Mean
				7	8	9	10	
Cultus Lake	May 5, 1923	153	1	–	1	–	–	8.0
Cultus Lake	May 30, 1942,	131–154	13	2	5	5	1	8.4
Holmes Creek	May 8, 1939	118–126	2	–	1	1	–	8.5
Holmes Creek	May 7, 1950	120–148	27	16	8	3	–	7.5
Total		118–154	43	18	15	9	1	7.8
Evans Creek	March–July,1929	93–114	8	8	–	–	–	7.0
Matthews Creek	April, 1931	80–141	2	–	–	2	–	9.0
Yakima River	July, 1930	135	1	1	–	–	–	7.0
Total		80–141	11	9	–	2	–	7.4
British Columbia		118–154	43	18	15	9	1	7.8
Washington		80–141	11	9	–	2	–	7.4
Total		80–154	54	27	15	11	1	7.7

TABLE IX. Body proportions (in percentages) of ammocoetes of *Lampetra richardsoni* from Millstone River, Nanaimo, B.C.

TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL
35	11.4	15.7	50.1	20.2
43	10.5	14.0	48.9	23.3
44	10.2	14.8	48.9	22.8
52	10.6	14.4	50.0	23.1
60	10.0	14.2	46.7	25.8
65	10.0	13.1	47.7	26.9
66	9.1	13.6	48.5	25.8
69	9.4	12.3	49.3	26.1
74	9.5	13.5	48.7	25.7
75	9.3	13.3	46.7	28.0
83	9.6	13.3	48.8	26.5
86	9.3	13.3	48.8	26.2
91	9.3	13.2	48.4	27.5
94	9.6	13.3	49.5	26.1
98	9.2	12.8	49.5	27.0
100	9.0	12.5	50.0	26.5
104	9.6	12.5	49.0	27.4

TABLE X. Body proportions (in percentages) of ammocoetes of *Lampetra richardsoni* from Evans Creek, Washington.

TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL
29	13.8	17.2	48.3	20.7
46	10.9	13.0	52.1	23.9
49	10.2	13.3	53.1	22.4
59	11.0	12.7	51.6	22.9
67	9.7	13.4	50.7	25.4
67	10.4	14.2	49.3	25.4
76	8.6	13.2	52.6	25.0
76	9.2	13.8	51.3	24.3
82	9.8	14.0	52.4	24.4
85	9.4	12.9	50.6	26.5
86	9.3	12.8	51.2	26.2
93	9.1	14.0	52.7	23.7
94	8.5	13.3	50.0	26.6
96	8.3	12.5	50.0	27.6
96	9.4	12.5	51.6	25.0
98	9.2	13.3	51.0	25.5
106	8.5	12.3	51.9	25.9
107	8.3	12.6	51.4	26.2
115	8.7	12.2	52.2	24.8
116	8.6	12.5	52.6	26.3
116	8.6	12.1	50.4	27.6
130	7.7	12.3	53.8	25.8

TABLE XI. Average body proportions (in percentages) of ammocoetes of *Lampetra richardsoni*, according to size, from two localities (from Tables IX–X). (E.C. = Evans Creek, Washington; M.R. = Millstone River, British Columbia.)

Locality	Group (*mm*)	Number of specimens	TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL
E.C.	20–29	1	29.0	13.8	17.2	48.3	20.7
M.R.	30–39	1	35.0	11.4	15.7	48.6	20.0
M.R.	40–59	3	46.3	10.4	14.4	49.3	23.1
E.C.	40–59	3	51.3	10.7	13.0	52.3	23.1
M.R.	60–99	11	78.3	9.5	13.3	48.4	26.5
E.C.	60–99	12	79.6	9.2	13.3	51.1	25.5
M.R.	100–04	2	102.0	9.3	12.5	49.5	26.9
E.C.	100–30	6	115.0	8.4	12.3	51.5	26.1

TABLE XII. Body proportions (in percentages) of larger ammocoetes of *Lampetra richardsoni* from Ames Lake, Washington.

Tag No.	TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL
3475V	95	8.4	12.1	53.1	24.7
3477V	98	8.2	13.3	51.5	25.5
3472V	99	8.1	13.1	51.5	23.7
3473V	100	8.0	13.0	53.0	24.0
3479V	104	8.7	13.0	52.4	24.5
3476V	113	8.0	11.9	52.7	25.6
3470V	119	7.6	12.6	52.9	25.2
3474V	120	7.5	11.7	54.2	25.0
3478V	127	7.9	11.8	52.0	26.8
3471V	128	7.8	12.5	51.6	26.6
Mean	110.3	8.0	12.5	52.5	25.2

TABLE XIII. Body proportions (in percentages) of larger ammocoetes of *Lampetra richardsoni* from North Creek, Washington.

Tag No.	TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL
3516V	102	9.3	13.2	48.5	27.5
3511V	113	8.8	12.4	51.3	26.5
3514V	114	8.7	12.7	50.0	27.2
3513V	115	9.1	13.0	49.1	27.0
3515V	115	8.7	12.6	50.0	27.0
3507V	118	8.5	12.7	51.7	25.8
3509V	119	8.4	11.8	52.9	26.1
3508V	120	8.3	12.9	52.1	25.8
3506V	121	8.3	12.4	51.2	26.4
3510V	121	8.7	12.4	50.8	26.4
3504V	127	8.7	12.6	51.2	26.4
3501V	128	9.0	12.5	51.2	25.8
3502V	129	8.5	12.4	52.0	26.0
3500V	137	8.4	13.1	51.5	25.9
3503V	137	8.4	12.8	50.4	27.4
Mean	121.1	8.7	12.6	50.9	26.5

TABLE XIV. Body proportions (in percentages) of larger ammocoetes of *Lampetra richardsoni* from Steel Lake, Washington.

Tag No.	TL (*mm*)	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL
3577V	86	8.1	11.6	52.9	25.0
3576V	124	7.7	11.3	52.4	27.4
3571V	132	7.6	11.4	52.3	27.2
3575V	132	7.6	11.4	53.8	26.5
3574V	133	7.5	11.3	53.8	26.3
3572V	144	7.1	11.9	55.0	25.5
3573V	152	7.3	11.7	54.0	27.2
3570V	175	5.5	8.7	55.5	27.8
Mean	134.8	7.3	11.2	53.7	26.6

TABLE XV. Average body proportions (in percentages) of larger ammocoetes of *Lampetra richardsoni* from five different localities (from Tables IX–XIV).

Locality	Number of specimens	TL (*mm*) Range	TL (*mm*) Mean	d–B_1 / TL	B_1–B_7 / TL	B_7–a / TL	a–C / TL
Millstone River	7	83–104	93.7	9.4	13.0	49.1	26.7
Evans Creek	13	85–130	102.9	8.7	12.7	51.5	26.0
Ames Lake	10	95–128	110.3	8.0	12.5	52.5	25.2
North Creek	15	102–137	121.1	8.7	12.6	50.9	26.5
Steel Lake	8	86–175	134.8	7.3	11.2	53.7	26.6
Mean	53	83–175	113.0	8.5	12.5	51.6	26.2

TABLE XVI. Variation in the degree of pigmentation on the tail and on parts of the head of larger ammocoetes of *Lampetra richardsoni*. (+ = weak pigmentation; ++ = moderate pigmentation; +++ = strong pigmentation.)

Locality	TL (*mm*)	Number of specimens	Tail			Upper lip			Subocular region			Prebranchial blotch			Branchial region		
			+	++	+++	+	++	+++	+	++	+++	+	++	+++	+	++	+++
Millstone River	83–100	4	–	4	–	–	4	–	1	–	3	1	2	1	2	1	1
Evans Creek	94–130	9	1	8	–	–	0	–	–	2	7	–	8	1	–	–	9
Ames Lake	95–128	10	5	4	1	–	10	–	–	–	10	2	6	2	2	5	3
North Creek	102–137	16	3	8	5	2	10	4	–	3	13	1	3	12	–	4	12
Steel Lake	86–175	8	1	2	5	–	8	–	–	–	8	–	–	8	–	–	8
Total	83–175	47	10	26	11	2	41	4	1	5	41	4	19	24	4	10	33
Percentage			21.3	55.3	23.4	4.3	87.2	8.5	2.1	10.6	87.3	8.5	40.4	51.1	8.5	21.3	70.2

Application of Mass Transfer Theory to the Problem of Respiration of Fish Eggs[1]

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ABSTRACT

The concentration of oxygen in the microenvironment of the developing fish egg is always less than the concentration in the supply water. Mass transfer theory is the theoretical tool for understanding the importance of the oxygen content and the velocity of the supply water as factors influencing oxygen uptake of fish eggs. The applicable literature on mass and heat transfer is reviewed and formulas for the calculation of oxygen depletion in the boundary layer surrounding the egg are suggested. Calculated dissolved oxygen requirements for eggs of two fish species at various water velocities are compared with experimentally determined dissolved oxygen requirements.

INTRODUCTION

The importance of oxygen availability for the normal development of fish eggs has been demonstrated by, amongst others, Hayes *et al.* (1951), Wickett (1954), Alderdice *et al.* (1959), Garside (1959), and Silver *et al.* (1963). The oxygen which is available to the egg is the dissolved oxygen at the egg surface. As the surface oxygen diffuses through the egg membrane, it is replaced from the supply water through a transfer process which combines diffusion with direct transport of oxygen by the water flowing over the egg. Diffusion is an essential part of the transfer process and this can occur only if there exists a concentration gradient in the water surrounding the egg. Thus the oxygen concentration at the egg surface is always less than the ambient concentration in the supply water, and the difference in concentrations depends on the rate of respiration by the egg and the velocity of the water. It is the oxygen content of the microenvironment of the fish egg which is biologically significant, but only the ambient oxygen content is readily accessible to measurement. The experimental studies cited show that hypoxia may result from low levels of ambient oxygen or low water velocities or a combination of both adverse conditions; that is, the observed limiting or critical levels of ambient oxygen are velocity-dependent. The experimental results could be more readily interpreted in terms of the corresponding limiting and critical levels in the microenvironment. What is required then is a quantitative theory of oxygen transport between the macro- and the microenvironment.

In an alternative approach to the problem of oxygen requirements, W. P. Wickett (unpublished data) has estimated the oxygen content in the microenvironment required to meet the full demands of the developing embryo. The calculation requires a knowledge of the normal oxygen concentration inside

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the egg, the normal oxygen uptake, and the diffusion constant for its passage through the egg membrane. Again a theory of oxygen transport through the supply water is required for calculating the corresponding ambient oxygen content, or for determining the minimum velocity for air-saturated supply water.

Thus a theory of oxygen transport is essential both for designing and interpreting experimental studies and for arriving at standards for the operation of fish hatcheries.

The problem of oxygen transport belongs to a general problem class referred to in the literature as mass transfer. A closely analogous problem arises in the study of heat transfer. In both classes of problems the transfer process takes place by a combination of diffusion and direct transport of solute, or heat, in the streaming fluid. Since the differential equations for mass and heat transfer have the same structure, theoretical or empirical solutions of the equations for heat transfer are applicable in mass transfer, after suitable re-interpretation of the mathematical symbols. The problem of mass and heat transfer to single spheres and packed beds of spheres has been studied both experimentally and theoretically in chemical and mechanical engineering. This paper reports and discusses the literature in these fields which is applicable to the respiration of fish eggs.

Symbols used in the equations of this paper are defined as follows:

N	Rate of transfer of solute to the sphere
R	Sphere radius
C_0	Solute concentration at sphere surface, assumed uniform over the sphere
C_1	Solute concentration outside solute boundary layer
C_e	Oxygen concentration inside egg capsule
k	Mass transfer coefficient
v	Carrier fluid velocity outside velocity boundary layer
ρ	Fluid density
μ	Fluid viscosity coefficient
D	Diffusion constant for solute in carrier fluid
D_e	Diffusion constant for oxygen in egg membrane
N_{Re}	Reynolds number
N_{Sc}	Schmidt number N_{Pr} Prandtl number
N_{Pe}	Peclet number
N_{Sh}	Sherwood number N_{Nu} Nusselt number
B	Coefficient in the Frossling equation
δ_v	Velocity boundary layer thickness
δ_s	Solute boundary layer thickness
K_b	Boltzmann's constant
T	Absolute temperature
a	Solute molecule radius

DIMENSIONLESS GROUPS

Before proceeding to discuss the literature it may be useful to give a statement of the problem in the terms commonly used in mass transfer theory. A solute concentration boundary layer is known to exist in the fluid surrounding a sphere, and solute is transferred across the layer at a rate proportional to the difference in solute concentrations on opposite sides of the layer. For transfer from the surrounding fluid to the sphere, the mass transfer coefficient, k, is defined by the equation:

$$N = 4\pi R^2 (C_1 - C_0) k. \quad (1)$$

The units of k are determined by the units used for the remaining quantities in the equation. For example, if R is expressed in cm, C_1 and C_0 in mg/cm^3 and N in mg/sec, then k is expressed in cm/sec. This form of stating the mass transfer relationships has the decided advantage that the thickness of the boundary layer and the distribution of solute within it, neither of which is generally known, do not appear in equation 1. Instead, the overall effect of a rather complex transport process is summarized in the mass transfer coefficient.

The mass transfer coefficient is combined with the sphere radius and the diffusion constant D (cm^2/sec) for the solute to form a dimensionless number, the Sherwood number:

$$N_{Sh} = 2kR/D. \quad (2)$$

The Sherwood number is a function of one or more of the following dimensionless numbers:

Reynolds Number

$$N_{Re} = 2 R\rho v/\mu; \quad (3)$$

Schmidt Number

$$N_{Sc} = \mu/\rho D; \quad (4)$$

Peclet Number

$$N_{Pe} = 2 R v/D. \quad (5)$$

Only two of these numbers are independent since the relationship

$$N_{Pe} = N_{Sc} N_{Re} \quad (6)$$

exists between them.

In carrying out numerical calculations with these formulas a consistent set of units, such as the c.g.s. system, is employed so that these numbers do have the correct dimensionless structure.

In the general case, the solute concentration gradient introduces a fluid density gradient which in turn gives rise to free convection of the fluid around the sphere. This process is described in terms of a fifth dimensionless number, the Grashof number. However, the free convection due to dissolved gas in water is entirely negligible compared with the forced convection so this number is omitted from the list. In what follows only mass transfer (or heat transfer) by forced convection is considered.

When mass transfer data are expressed in terms of these dimensionless numbers, or groups, it is possible to correlate data from geometrically similar but otherwise dissimilar situations. Thus, if a relationship

$$N_{Sh} = f\,(N_{Re}, N_{Sc})$$

is found within a given range of N_{Re} and N_{Sc} for the mass transfer of one solute in a given fluid, the same relationship holds for mass transfer of any other solute in a different fluid, provided that the situation is geometrically similar and that the Schmidt and Reynolds numbers fall within the given range. Further, as Ranz and Marshall (1952) have pointed out, the analogy between heat and mass transfer requires that the same functional relationship holds for heat transfer when the Sherwood number and Schmidt number are replaced by their respective analogues. The analogue of the Sherwood number is the Nusselt number, N_{Nu}, and the analogue of the Schmidt number is the Prandtl number, N_{Pr}. In mass transfer theory the Nusselt number is frequently used interchangeably with the Sherwood number.

An extensive list of the dimensionless numbers used in mechanical and chemical engineering is given by Boucher and Alves (1959).

MASS TRANSFER TO SINGLE SPHERES

The transfer of solute between a sphere and the surrounding fluid is defined, in principle, by a pair of partial differential equations, the Navier-Stokes equation for the fluid velocity and the diffusion equation for the solute transport in the velocity field. Unfortunately no exact analytic solutions for the problem have been found, primarily because of the non-linear character of the Navier-Stokes equation. For small values of the Reynolds number, Stokes' approximate analytic solution of the Navier-Stokes equation can be used and the diffusion equation can then be solved numerically. Friedlander (1957) used an approximate boundary layer theory based on the diffusion equation and obtained the Sherwood number as a numerical function of the Peclet number. Yuge (1956) solved the diffusion equation numerically for a smaller range of Peclet number and, within the range covered, his results agree with those of Friedlander. Both authors used Stokes' solution for the velocity field.

The validity of Friedlander's solution therefore depends on the accuracy of Stokes' solution for the velocity field. Stokes' solution is generally believed to be reliable for Reynolds number less than 1, but the magnitude of the error at any given Reynolds number is not known with any degree of certainty. However, the sign of the error can be determined. An examination of the effect of the neglected terms in the Navier-Stokes equation does indicate that Stokes' solution predicts a velocity in the boundary layer which is less than the true velocity so that Friedlander's solution underestimates somewhat the amount of mass transfer.

Friedlander's solution can be expressed conveniently in the form of equation 7, where A is a slowly varying function of N_{Pe} given in Table I:

$$N_{Sh} = 0.89 \, A \, N_{Pe}^{1/3}. \quad (7)$$

It is applicable over a wide range of Peclet number but only for Reynolds numbers less than 1. For chum salmon eggs, for example, the maximum supply water velocity for which this solution can be used is about 60 cm/hr.

TABLE I. Correction factor for equation 7.

N_{Pe}	A
10	1.64
30	1.40
100	1.23
300	1.16
10^3	1.10
10^4	1.04
10^5	1.00

At higher Reynolds numbers no complete solutions of the differential equations are available. The boundary layer theory of Prandtl and Blasius (see Chapman, 1960) shows that the Sherwood number is a function of the Reynolds and Schmidt numbers of the following form:

$$N_{Sh} = 2.0 + B \, N_{Re}^{1/2} \, N_{Sc}^{1/3}, \quad (8)$$

where B is a number which is usually found by experiment because of the complexity of the calculations necessary to evaluate it.

The physical meaning of this equation is clarified by a consideration of the velocity and solute boundary layers surrounding the sphere. Close to the sphere the streaming fluid flows approximately parallel to the spherical surface; it has zero velocity at the surface and a steep velocity gradient in the velocity boundary layer. At the outer surface of this boundary layer the fluid velocity approaches its bulk value. The effective thickness, δ_v, of the boundary layer is given in this equation,

$$\delta_v \approx 2 \, R/N_{Re}^{1/2}, \quad (9)$$

for a sphere of radius R. Within this boundary layer there is a solute boundary layer, in which solute is transferred radially inward by diffusion and transported tangentially over the sphere by the streaming fluid. At the sphere surface, diffusion dominates; at the outer surface of the boundary layer, direct transport dominates and the solute concentration has approximately its bulk value. The solute boundary layer has an effective thickness δ_s:

$$\delta_s \approx \delta_v/N_{Sc}^{1/3}, \quad (10)$$

or, from equation 9,

$$\delta_s \simeq 2R/N_{Re}^{1/2} N_{Sc}^{1/3}. \quad (11)$$

The Sherwood number by definition expresses the rate of diffusion across this boundary layer; hence,

$$N_{Sh} = 2R/\delta_s, \quad (12)$$

or, by equation 11,

$$N_{Sh} \simeq N_{Re}^{1/2} N_{Sc}^{1/3}. \quad (13)$$

Thus the coefficient, B, in equation 8, the Frössling equation, is expected to be of the order unity. The additive constant, 2.0, represents the contribution to the solute transfer from diffusion parallel to the streaming fluid, which was neglected in the above discussion.

The coefficient, B, is generally treated in the literature as a constant, independent of N_{Sc}, but Pohlhausen (1921) showed that B does vary somewhat with N_{Sc} in the case of heat transfer from a flat plate. For small N_{Sc}, B is expected to increase with increasing N_{Sc} but approach some limiting value for very large N_{Sc}.

Frössling (1938) measured the rate of evaporation of falling liquid drops in air and obtained a value for B of 0.55 for $2 < N_{Re} < 1000$ and $0.6 < N_{Sc} < 2.6$. Ranz (1952) correlated a large set of mass and heat transfer data, using equation 8, with a value of 0.60 for B. The range of N_{Sc} is $0.60 < N_{Sc} < 380$, but the data are heavily weighted toward the smaller values of N_{Sc}; the range of N_{Re} is $1 < N_{Re} < 70{,}000$.

Axel'rud (1953) measured the rate of dissolution of solid spheres of KCl and KNO_3 in water, for which N_{Sc} is of the order 800, and obtained a B value of 0.82 in the range $600 < N_{Re} < 2600$. Garner and Suckling (1958) measured rate of dissolution of solid spheres in water, supported in a transparent cylinder, and found a B value of 0.95 for N_{Sc} values of 1100 and 1340 and N_{Re} in the range $60 < N_{Re} < 600$. The Reynolds number used in this correlation was the average number for the non-uniform velocity over the pipe cross-section. A correction to B can be estimated from the mean Reynolds number for the data correlated, using the authors' fig. 5. The estimated corrected value of B is 0.86. Griffith (1960) measured the rate of dissolution in water of liquid drops of a number of substances and found a mean value for B of 0.67 for $1000 < N_{Sc} < 1400$ and $4 < N_{Re} < 120$.

A more extensive bibliography of literature on mass transfer to single spheres is given by Steinberger and Treybal (1960).

The expected increase of B with N_{Sc} is supported by the available experimental data. Moreover, Friedlander's solution joins on quite well to equation 8 at Reynolds numbers of the order unity when the relevant values of B and N_{Sc} from Frössling, Ranz and Axel'rud are used. At a Reynolds number of 1, the Sherwood numbers calculated by both formulas agree within 10%. Griffith's formula intersects Friedlander's solution at a Reynolds number of 4, where the latter is expected to predict too small a Sherwood number. This suggests that

B is higher than 0.67 for N'_{Sc} of the order 1000 and that greater weight should be given to Axel'rud's value of 0.82.

For oxygen transport in water, N_{Sc} is of the order 1000 and the best estimate of B, rounded to one significant digit to indicate the probable accuracy, is 0.8.

A more reliable mass transport formula could be obtained, if required, by solving the Navier-Stokes equation and then the diffusion equation with the help of an electronic computer. Jenson (1959) has obtained some numerical solutions, by manual calculations, of the Navier-Stokes equation for the velocity field. The relaxation method used is convenient for use with an electronic computer, although it requires a fairly large capacity memory unit. Once the velocity field has been obtained for a suitable range of Reynolds numbers, the diffusion equation can be solved for the required Schmidt numbers by a similar relaxation method. A substantial compression in the size of the arrays of numbers which would have to be handled could probably be achieved by expanding the field variables in spherical harmonics. The problem then simplifies to the solution of a set of ordinary differential equations for the expansion coefficients.

MASS TRANSFER TO PACKED BEDS OF SPHERES

No analytical solutions for mass transfer in packed beds of spheres are known but Ranz (1952) has suggested that the mass transfer to each sphere in the bed can be described by an equation like equation 8 but with N_{Re} replaced by an effective Reynolds number N'_{Re} based on the mean fluid velocity through the voids between the spheres. For single- or multiple-layer packed beds in which the carrier fluid flows normal to the layers, N'_{Re} is $K\ N_{Re}$, where N_{Re} is calculated from equation 3 using the superficial velocity, and $1/K$ is the fraction of the cross-sectional area not obstructed by the spheres. An idealized model suggests that K should be 10.73. Ranz then shows that the mass transfer data of Gamson *et al.* (1943) for packed beds can be correlated by the equation

$$N_{Sh} = 2.0 + 0.60\ N'^{1/2}_{Re}\ N_{Sc}^{1/3},$$

with $N'_{Re} = 9.1\ N_{Re}$.

The Schmidt number is of the order unity and N'_{Re} is in the range $100 < N'_{Re} < 1200$. The same void factor of 9.1 also explained the measured pressure drop through the packed bed when the pressure drop was assumed to be additive for each sphere, and the effective Reynolds number, N'_{Re}, was again used in calculating the pressure drop from an individual sphere. Thus the use of an effective Reynolds number, equal to $9.1\ N_{Re}$ for close-packed beds, is substantiated by two independent sets of data.

For oxygen respiration in close-packed beds of fish eggs, with the supply water perfusing through the bed, the appropriate transport formula is

$$N_{Sh} = 2.0 + 0.8\ (9.1\ N_{Re})^{1/2}\ N_{Sc}^{1/3}, \qquad (14)$$

which should be applicable, with the same degree of reliability as equation 8 for single eggs, for N_{Re} greater than 1. In using this formula, the oxygen depletion of the supply water as each layer of the bed is traversed must be taken into account. If the packed bed does not conform to the close-packed tetrahedral pattern the method described by Ranz for calculating the effective Reynolds number from the measured pressure drop in the bed should be useful.

DIFFUSION CONSTANT FOR DISSOLVED OXYGEN

Values of D for oxygen in water have been listed by Millington (1955). The temperature range covered does not include temperatures frequently required for the development of fish eggs, the quantity $D\mu/T$ should be a constant, and simple extrapolation of the data is unlikely to be reliable. According to the Stokes-Einstein equation for the diffusion of small particles (see Treybal, 1951),

$$D = K_b T/6\pi a\mu. \tag{15}$$

Table II lists Millington's data together with the calculated values of $D\mu/T$. The constancy of $D\mu/T$ is confirmed for four of the six values listed. The average value of $D\mu/T$ for these four values of D is 0.720×10^{-9} g-cm/sec^2-deg. Thus

$$D = 0.720 \times 10^{-9}\ T/\mu\ \text{cm}^2/\text{sec}, \tag{16}$$

when μ is expressed in g/cm-sec.

TABLE II. Diffusion constant for oxygen in water.

Temperature	D	$D\mu/T$
°C	cm²/sec	g-cm/sec²-deg
16	1.875×10^{-5}	$.720 \times 10^{-9}$
18	1.98	.719
18.2	1.99	.721
22	2.22	.721
25	2.38	.714
25	2.16	.647

CALCULATION OF OXYGEN DEPLETION IN THE BOUNDARY LAYER SURROUNDING CHUM SALMON EGGS

As an example of the application of the mass transfer theory, the difference in oxygen concentrations across the boundary layer is calculated using data taken from Alderdice *et al.* (1958) for the respiration of chum salmon eggs.

At the incubation temperature of 10°C (283°K) the diffusion constant from equation 16 is

$$D = \frac{.720\ (283)\ 10^{-9}}{(1.308)\ 10^{-2}} = 1.56 \times 10^{-5}\ cm^2/sec$$

$$v = 85\ cm/hr = 2.36 \times 10^{-2}\ cm/sec$$

$$N_{Re} = \frac{(2R)\ v\ \rho}{\mu} = \frac{(0.74)\ (2.36)\ (1.00)\ 10^{-2}}{(1.308)\ 10^{-2}} = 1.33$$

$$N_{Sc} = \frac{\mu}{\rho D} = \frac{(1.308)\ 10^{-2}}{(1.00)\ (1.56)\ 10^{-5}} = 836$$

$$N_{Sh} = 2.0 + 0.8\ N_{Re}^{1/2}\ N_{Sc}^{1/3} = 10.6$$

$$k = \frac{N_{Sh}D}{2R} = \frac{10.6\ (1.56)\ 10^{-5}}{0.74} = 2.24 \times 10^{-4}\ cm/sec$$

$$N = 5.21 \times 10^{-3}\ mg/hr \text{ at } 452 \text{ degree-days;}$$

$$= 14.5 \times 10^{-7}\ mg/sec$$

$$C_1 - C_0 = \frac{N}{(4\pi R^2)k} = \frac{(14.5)\ 10^{-7}}{(1.72)\ (2.24)\ 10^{-4}} = 3.76 \times 10^{-3}\ mg/cm^3.$$

When C_1 was 10.14 ppm, 20 out of 20 eggs hatched normally. The corresponding concentration of oxygen at the egg surface was:

$$C_0 = 10.14 - 3.76 = 6.4\ ppm.$$

The calculation illustrates the fact that the oxygen concentration drop across the boundary layer can amount to a significant fraction of the oxygen content of the supply water in equilibrium with air, at velocities as high as 85 cm/hr.

APPLICATION OF THEORY TO EXPERIMENTAL DATA ON SALMONID EGGS

The dissolved oxygen concentration required, at any given supply water velocity, to meet the full demands of the fish embryo can be calculated from the mass transfer formulas if the following additional data for the egg are available: the oxygen uptake, N; the residual oxygen concentration, C_e, inside the capsule; the diffusion constant, D_e, for oxygen passage through the capsule membrane; and the membrane thickness, δ. Wickett (unpublished data) has provided estimated values of these quantities for steelhead trout and chinook salmon under conditions of oxygen sufficiency. They are listed in Table III.

From these data and the mass transfer formulas, the corresponding dissolved oxygen concentration was calculated at a number of water velocities. The calculated curves, which are applicable to well-separated eggs, are shown in Fig. 1. It is worth noting that the curves become rather flat for high water velocities, so that a further increase of velocity beyond some point is ineffective in practice in reducing the oxygen requirement.

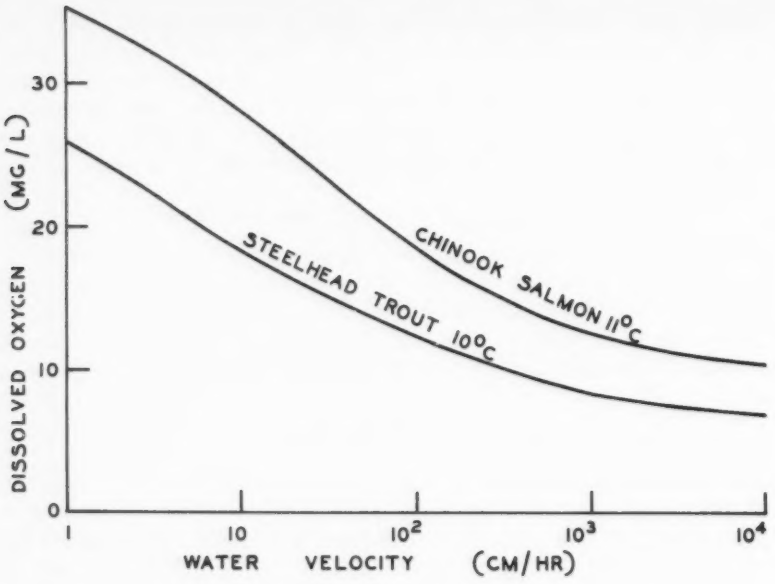

FIG. 1. Calculated dissolved oxygen concentration required to provide full oxygen uptake by two species of fish embryos. Abscissa is the apparent water velocity for well-separated eggs.

Table 3 of Silver *et al.* (1963) indicates that the length of hatched steelhead trout fry incubated at 11.2 mg/liter oxygen decreases slightly when the water velocity ranges from 740 to 34 cm/hr, and thereafter more rapidly. The lower curve of Fig. 1 indicates oxygen sufficiency with 11.2 mg/liter down to 175 cm/hr and a deficiency of 3.5 mg/liter at 34 cm/hr. Table 7 of Silver *et al.* shows that hatched chinook salmon fry incubated at 11.7 mg/liter oxygen showed increased length with increased velocity up to 1350 cm/hr, the maximum velocity used. Figure 1, upper curve, predicts a higher velocity of 2000 cm/hr for this oxygen content.

TABLE III. Data used to calculate dissolved oxygen requirements of Fig. 1.

Quantity	Estimated value	
	Steelhead trout	Chinook salmon
C_e	3.0 mg/liter	3.0 mg/liter
N	2.0×10^{-6} mg/sec	4.1×10^{-6} mg/sec
D_e	0.18×10^{-5} cm²/sec	0.18×10^{-5} cm²/sec
δ	0.004 cm	0.006 cm
R	0.32 cm	0.41 cm
C_0 (calc)	6.5 mg/liter	9.5 mg/liter

TABLE IV. Values of C_e and N used to calculate curves of Fig. 2.

Fry length	C_e	N
mm	*mg/liter*	*mg/sec*
19	2.7	0.83×10^{-6}
18	2.0	0.50
17	1.6	0.34

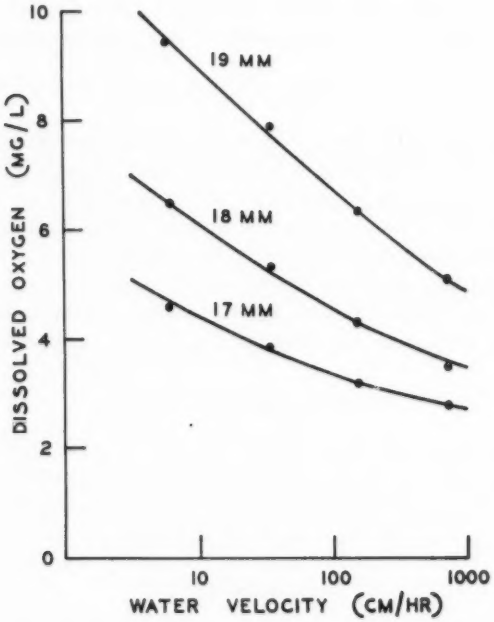

FIG. 2. Dissolved oxygen concentrations at various water velocities required to produce steelhead trout sac fry of various lengths. Curves calculated from assumed values of extracellular oxygen concentration and oxygen uptake (Table IV). Experimental points from Silver *et al.*, 1963.

The dissolved oxygen requirements calculated for the assumed conditions of oxygen sufficiency thus agree in a general way with the experimental data, but a more critical test of the theory can be proposed. Figure 4 of Silver *et al.* (1963) shows how the mean length of steelhead trout sac fry depends on both dissolved oxygen and water velocity. It is reasonable to assume, as a first approximation, that sac fry of equal length experienced equal concentrations

and consumed equal amounts of oxygen. To investigate this assumption, values for the internal oxygen concentration, C_e, and the uptake, N, were found for each length of sac fry which provided the best fit to the experimental data. The assumed values of C_e and N are listed in Table IV. The dissolved oxygen concentration was then calculated as the sum of C_e, the concentration drop across the capsule membrane and the drop across the boundary layer.

Figure 2 shows the calculated curves for three sizes of sac fry together with the data interpolated from fig. 4 of Silver *et al.* In each curve the four experimental points are fitted quite well, using the two adjustable quantities, C_e and N. Thus the mass transfer theory together with the assumption of equivalent microenvironments for eggs producing similar sac fry makes it possible to infer the internal oxygen content of the egg and its uptake. Independent measurements of these quantities should, if possible, be made to verify the theory.

CONCLUSION

The use of mass transfer theory provides an appreciation of the dynamical relation between the developing fish embryo and its aquatic environment. Because the egg acts as an oxygen sink, the oxygen level at the egg surface may be much less than that of the water supply even at high water velocities. The limiting values of dissolved oxygen and water velocity are not simple constants but are interrelated and related to the temperature-dependent oxygen uptake and the egg diameter. The theory appears to describe the physical environment with sufficient accuracy to provide for useful biological interpretation.

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Significance of Early Emergence, Environmental Rearing Capacity, and Behavioral Ecology of Juvenile Coho Salmon in Stream Channels[1]

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ABSTRACT

Coho behavior was examined in two glass-walled stream channels containing riffle-pool series. The apparatus permitted volitional residence. Experimental groups of coho in each channel were permitted to emerge from a simulated redd environment and subsequently studied for 5 months.

Aggressive behavior in these coho fry was initiated within 1 week of emergence from the gravel. Within 10 days of emergence, coho occupied and defended feeding territories well-distributed in riffles. Initial aggression was nipping and chasing but within 2 weeks of emergence, aggression also involved threat.

Pools appear to constitute principal security features of coho environment. A behavioral pattern termed "fright huddle" was observed and described. Individual fish developed habitual living patterns in the channels, and smaller fish tended to occupy downstream areas.

Earliest-emerging coho enjoyed ecological advantages over later-emerging fish. The former were larger at a given time and had a greater tendency to remain in stream channels, suggesting that they have "settler's rights" to available environment and/or better feeding opportunity. The results are discussed with reference to environmental rearing capacity and volitional residence.

INTRODUCTION

The fry of stream-dwelling salmonids must migrate out of the gravel in which they were deposited as fertilized eggs. Little is known of this initial migratory phase of the general life history in terms of the mortality attributable to it or of behavioral mechanisms. The possibility of migrational delay — either by the individual fry or by the redd population to which it belongs — and the ecological significance of such delay have received scant attention. Similarly, the question of trophic relations, onset of feeding behavior, and the host of possible circumstances capable of influencing intra-gravel movement and migration have been little investigated.

It is well known that the rate of emergence and length of the emergence period may vary considerably in both natural redds and in laboratory facsimilies. Apart from the factors responsible for the nature of emergence from the gravel, the ecological significance of a particular position on the emergence time scale is presently unknown for both the individual fry or its racial population.

First-emerging coho salmon fry (*Oncorhynchus kisutch*) move downstream in small numbers soon after emergence. Whether this movement is due to an innate migratory urge or to displacement by the current is not known but

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Chapman (1962) has reported that this "drift" occurs despite an apparent abundance of suitable living space at the stream margins.

Chapman (1962) has shown that aggressive behavior is an important cause of downstream movement of coho fry prior to the smolt migration. Aggression in juvenile coho is associated with intraspecific competition for food and shelter. Our present experiment is one of a series concerned with the behavioral ecology of juvenile coho salmon. The primary objectives were to evaluate possible significance of early emergence, to establish when aggression and territoriality develop in newly emerged coho fry, and to attempt measurement of the relative roles of pool and riffle environments, in terms of rearing capacity, for the juveniles of this species. The study extended from February to early July 1963.

DESCRIPTION OF THE APPARATUS

Two glass-fronted wooden stream channels, 25 ft (7.62 m) long and 3 ft (0.91 m) in both width and height, located at the Alsea Fish Hatchery (Oregon State Game Commission) on the North Fork of the Alsea River in Western Oregon, were used for the study. These channels were positioned side by side with approximately 5 ft between them to facilitate construction of a median observation enclosure (Fig. 1). Rapid observation of both systems with minimal disturbance could be made from a central bench.

The water supply was tapped from the main line entering the hatchery from a collection system located some 400 yards (366 m) upstream at a concrete dam across the river. The flow was directed into each system through aluminum irrigation pipe and controlled by separate valves. The outflow from each channel passed through an inclined-plane trapping system into a V-notch 90-degree weir forebay. Both weirs were calibrated so that identical flows

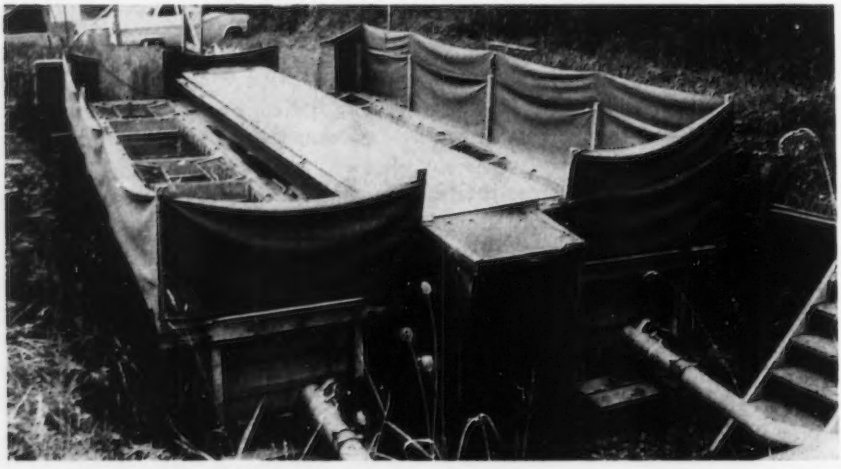

FIG. 1. Paired observation channels. Channel walls facing central observation chamber are glass.

could be maintained in both systems and a constant flow of 0.45 ft^3/sec (12.44 liters/sec) was passed through each channel during the present experiment.

In each channel, an alternating series of riffles and pools was constructed using plywood floorboards. The left channel contained three riffles and three pools, each 4 ft (1.21 m) in length, while the right channel was composed of two riffles, each 8 ft (2.43 m) in length, and two pools of 4 ft (1.21 m) in length (Fig. 2). Thus, the two channels differed in the relative amounts of each bottom type present, the left system containing 50% more pool area but 25% less riffle area than did the right system.

The flooring was constructed to allow 5 inches (12.7 cm) of water depth over the riffle boards and 18 inches (45.72 cm) over the pool boards. Both riffles and pools were overlain with gravel between 1 and 2 inches (2.54–5.08 cm) in greatest diameter. At the constant flow rate of 0.45 ft^3/sec (12.44 liters/sec) the average riffle velocities ranged from 0.8 to 0.9 ft/sec (0.24–0.27 m).

The upstream end of each channel was screened and resident fish were able to leave the system only by passing out through wall slots in the downstream end of the channel into the inclined-plane trap.

During the experimental period, maximum and minimum mean weekly temperatures based on daily records ranged from 45.1 to 57.9°F (3.23–14.37°C) and from 42.9 to 54.5°F (6.05–12.43°C), respectively.

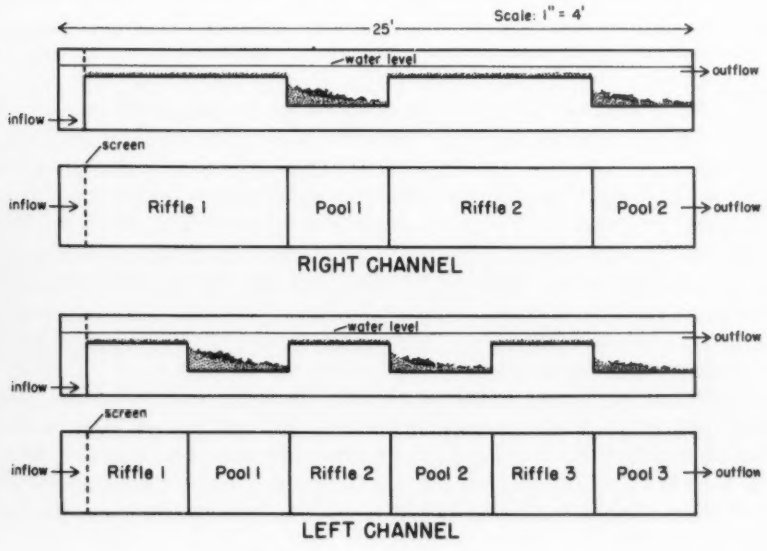

FIG. 2. Diagrams showing side and top aspect of each channel.

METHODS

Fertilized eggs from Fall Creek[3] adults (two males × one female) were incubated in standard hatchery baskets in laboratory facilities at Oregon State University until well into the sac fry stage. On February 22, 1963, the sac fry were transported to the stream channels and 300 fry were installed in each system within a nontoxic plastic screen container. A 6-inch (15.24-cm) layer of gravel was carefully placed over the fish in the containers so that to escape the container, the fry had to migrate up through the layer of gravel. One of the containers was suspended in the first pool of each channel against the far wooden wall. Emergence from the gravel into the pools simulated the natural environmental experience of coho fry except that the initial postemergence period was spent in pools rather than among the spawning rubble of the natural stream bed.

The first 25 fish to emerge in the left system and the first 46 fry in the right system were fin-clipped for future identification by removal of the right ventral fin. Fry leaving the channels entered the traps, were measured for fork length, and were released into the Alsea River. Residence was maintained strictly on a voluntary basis to approximate as closely as possible behavior in the natural stream environment. Juvenile coho behavior was observed for a period of some 4 months prior to termination of the experiment in early July.

RESULTS

Initiation of Aggression and Territoriality

The first fry emerged on March 7 in the left channel, and in the right channel between March 7–10. By March 15, the emergence was well underway in both systems and the first fry were taken in the traps. On March 13, about 40 fry were observed feeding vigorously in the first pool of the right channel. No aggression was detected in this group during a 10-min observation period, but six nips were recorded among some 50 fry in this pool during a similar period 2 days later. On the latter date the first fry were seen feeding actively on the riffle areas in the right channel, however, they were few in number, the majority remaining in the pools.

By March 19, fry in the left channel had invaded the riffles and were present in large numbers in the first riffle, well-scattered and exhibiting frequent aggression in the form of threat, nipping, and short drives and chases involving adjacent individuals. Little sign of aggression was observed in the pools at this time. Similar observations were recorded on March 26.

On March 29 freshet conditions in the main stream turned the water quite turbid, much food was present in the drift, and the fry were actively feeding throughout both systems. Aggressive behavior was frequent in the riffle areas when the fish could be seen. Variably turbid water conditions prevailed during the first week of April but territorial behavior in the riffles was

[3]Fall Creek is a tributary of the Alsea River, a stream entering the Pacific Ocean near Waldport, Oregon.

frequent when observable. Riffle-invading fry pursued a characteristically zig-zag course up through the riffle as they entered specific territories and were driven from them by their aggressive owners. Unless an empty "slot" could be found or unless territorial fish were successfully displaced, such invaders would turn and flee downstream into the pool.

From those general observations, it appears that aggressive behavior in coho fry begins within 1 week of emergence from the gravel. At this time, the invasion of riffle areas in these artificial streams coincided with the appearance of aggressive interactions amongst coho fry in the pools. Within 10 days after appearance of emergent fry in the channels, large numbers had invaded the shallow riffle areas, occupying and defending feeding territories well distributed throughout individual riffles. When aggressive behavior was initially observed in the pool, it consisted of simple nipping and chasing but aggression in the riffles 4 days later entailed threat, nipping, driving, and chasing amongst adjacent individuals. Aggression in the riffles was particularly directed toward fry which were invading the riffle areas and, by doing so, trespassing in territories defended by resident coho.

General Characteristics of Aggressive Behavior

The behavior patterns of juvenile coho were discussed by Chapman (1962). Nipping, chasing, flight, fighting, territorial defense, redirected aggression, dominance posturing, lateral display, and parallel swimming were commonly seen in the channels. Formation of hierarchies could not be confirmed due to the large numbers of coho present in the pools but the general size-dominance relationship usually determined the outcome of social interactions between individuals. Neither hiding nor submission was observed in the channels. The relative size and uniformity of the environment may have discouraged expression of the former behavioral pattern and it is possible that submission is a behavioral artifact induced by the close confines of small aquaria wherein adequate escape opportunities are not provided.

To provide some basis for interpreting the significance of aggressive behavior in the artificial streams, nip intensity was recorded in the pools and riffles. As previously indicated, the first signs of aggressive behavior were observed within 1 week of first emergence on March 7 (Table I).

Table I. Changes in level of aggression during the study period.

Date	No. of fish		No. of 10-min observation periods	Observation time limits	Level of aggression (nips/fish/10 min)		
	In channels	Observed			Mean	Range	Confid. limits ($t_{.95}$)
March 13	55	30	1	0915–0925	0.0		
" 15	60	50	1	1316–1326	0.12		
April 18	210	105	3	0900–0930	0.53	0.08–1.33	0.0–2.21
May 4	287	235	10	0900–1100	0.78	0.0–2.00	0.33–1.24
" 11	281	110	10	1010–1200	2.64	0.0–5.00	1.44–3.83
" 20	241	93	10	1000–1150	7.85	0.0–21.00	3.20–12.50
" 30	155	60	4	1015–1120	4.67	4.0–6.00	1.09–8.24

A trend toward increasing aggression may be seen, which is accelerated in early May. When the data were pooled within the two time-intervals, March 13–May 4 and May 11–May 30, and subjected to a *t*-test, the resulting mean aggression levels in the latter period were significantly higher ($P = .05$). The rise in level of aggression paralleled the rate of egress from both channels and rising water temperature. When aggression level was plotted against the daily rate of outmigration during the 7-day period bracketing the day whereon aggression was measured, there appeared to be a positive correlation between these two variables but the relationship was not statistically significant. The correlation between aggression and temperature level was a positive and significant one ($P = .05$).

The levels of aggression in riffle and pool environments are not strictly comparable since the relative intensity of interaction does not appear to be equal in both areas. Riffle interactions are more violent, involve higher degrees of locomotor activity due to the additional current velocity, and more often culminate in a chase→near-contact nip or chase→contact nip than do pool interactions. The latter involve less active chasing and nipping except in the case of prolonged territorial disputes. In addition, pool interactions are associated with more lateral threat, circling, parallel swimming, and low level dominance posturing. A more complete analysis of the behavioral differences in both environments will be reported in a later paper. Hartman (1963) demonstrated in *Salmo trutta* that higher water velocities produced greater nipping relative to displaying, and lower water velocities produced greater displaying.

Juvenile coho exhibit intense fighting behavior which appears to be associated primarily with attempts to set up or maintain territories in pools. Rapid circling and severe nipping attempts concentrated at the caudal region typify this behavior. Whereas fighting was commonly seen in the channels during the spring, its frequency declined as the season progressed until in May, when the coho averaged about 45–48 mm in total length, its occurrence was quite rare. The last fight observed took place on May 11 between two small coho and pertained to a territorial dispute in the last pool of the right channel. The average length of the 16 coho in this pool was 45.6 mm on May 1. The full fighting pattern was not commonly observed following initial encounters within small populations of *Lepomis cyanellus* (Greenberg, 1947) and *Salmo gairdneri* and *Salvelinus fontinalis* (Newman, 1956).

Fright Huddle

When juvenile coho are badly frightened, either purposely by the observer, or due to natural events such as the overflight of birds and avian activities in the system[4], a predictable sequence of events is initiated. Coho occupying territories in the riffles flee rapidly into their "home" pool, assuming a quiescent position on or near the bottom and adjacent to the glass wall. If the disturbing influence is extended to the pool, all fish move into close contact with one

[4]Water ousels (*Cinclus mexicanus*) sometimes visited the channels and foraged in the riffles.

another near the bottom of the pool, forming a close grouping. This fright behavior is best described as huddling and the consequent grouping is termed the "fright huddle". It has been elicited in a smaller stream channel containing groups of 10–20 coho.

Physical contact both with the bottom and with adjacent individuals, rapid respiratory movements, complete lack of aggression, and maintenance of position by slight fin movements characterize this group-behavioral reaction in the pools. Breakup of the huddle commences when a few individuals at the periphery make short drives to and from the huddle, usually toward the surface, and engage in short-term and low-intensity aggressive behavior directed at close companions. The huddle slowly expands and may reach dimensions two to three times that of its original size before an appreciable number of coho venture from it.

By the time the huddle has lost its identity as a close grouping of fish, the more aggressive coho are actively feeding and behave as they did prior to the disturbance. It should be added that smaller coho which usually leave the huddle first are also first to re-invade the riffles but their territorial opportunities are terminated by the larger fish when the latter begin feeding again.

Huddling behavior can be elicited in riffle conditions of rapid flow and shallow water by carefully introducing groups of coho from a container. The great majority of fish move to the glass wall and maintain position in the current, forming an elongated but compact grouping which preserves its identity for 10–20 min before any aggression returns. The few fish not joining the huddle swim rapidly downstream into the pool. Breakup of the riffle huddle is signified by a "stringing-out" in a downstream direction, precipitated when the upstream members of the group initiate low intensity dominance posturing which increases in vigor, accompanied by driving and nipping at nearby companions.

The fright reaction involves contact with the substrate and adjacent individuals and, in rapid flow, maintenance of position against the current. When coho feeding in the riffles are disturbed, their low level fright reaction is flight into the pools.

Social Habit, Territoriality, and Social Stability

To measure the stability of the distribution in the stream channels, we recorded changes in location of individual fish. Fin clips and differences in coloration, fin shape, body size, and behavior made it possible to identify individual fry.

Over the period of 1 week, the fish in the right channel were closely observed for individual characteristics. Of the 60 coho living in the system, 10 fin-clipped fish and 3 unmarked coho were selected for further study. These 13 fish could be recognized with complete reliability within the population. They were healthy, active fish which reflected the range in size encountered in the population and gave no indication that they were in any way abnormal or aberrant in behavior. The coho were closely observed during the daylight hours on 3 days, June 23,

25, and 27. The position and general activity of each fish was recorded at least once per hour and far more often during the early morning and late evening periods. The position data are compiled in Table II.

Position changes averaged one per fish per 24 hr of aggregate observation time. One individual never moved and five moved only once. Ten of 13 coho showed definite constancy of preference for one riffle or one pool. Travel in the system was confined to a pair of adjacent pool-riffle units. Larger coho (7 fish of 80–90 mm) chose positions along the channel walls in the uppermost pool and riffle and tended to remain there. None was seen in the second riffle or pool. Smaller coho (6 fish of 70–75 mm) spent their riffle time in riffle 2 but their pool time in pool 1, wandered in position more than the larger fish while in the riffle, and tended to take a central position in pool 1. Even though well distributed along riffle 2, they were never seen in pool 2.

TABLE II. Percentage of time 13 coho spent in riffle or pool positions during late June 1963.

	June 23		June 25		June 27		Total	
Fish	Pool	Riffle	Pool	Riffle	Pool	Riffle	Pool	Riffle
1	8.1	91.9	81.6	18.4	100.0		60.9	39.1
2		100.0	13.8	86.2		100.0	8.4	91.6
3		100.0	12.2	87.8	100.0		18.0	72.0
4	79.4	20.6	No record		61.1	38.9	73.0	27.0
5	100.0		92.6	7.4	100.0		78.3	21.7
6	100.0		100.0		100.0		100.0	
7		100.0	2.7	97.3		100.0	1.2	98.8
8		100.0		100.0		100.0		100.0
9		100.0	29.3	70.7	16.7	83.3	19.0	81.0
10		100.0	40.5	59.5	22.7	77.3	30.7	69.3
11	100.0		100.0		37.5	62.5	87.6	12.4
12	100.0		61.5	38.5	100.0		87.8	12.2
13	No record			100.0	51.0	49.0	24.3	75.7

Individual coho are prone to develop habitual patterns of living within the system. These patterns fall into two general categories involving primary association either with a particular riffle or pool, at least during the daylight hours. Both categories are strongly identified with territoriality. As coho preferring riffle association flee into the pools when frightened, the pools form the basic security orientation for all fish in the system.

DISTRIBUTION PATTERN IN THE CHANNELS

The coho fry emerged from the gravel into the uppermost pool of each channel. Their post-emergence distribution involved an initial radiation in both upstream and downstream directions (Table III). Main movement out of pool 1 took place between March 15–19 and was in a downstream direction in the left system but in an upstream direction in the right system. The possibility exists

that an inequality in flow created this discrepancy as the channels were not calibrated at this time. But the impetus for distribution throughout the channels is taken to be the rapid accumulation of fry in the uppermost pools.

Although a few fry passed out of both channels during the first week, their numbers were but a small fraction of the resident populations. Rate of emigration increased following March 19, remaining high until March 26 in the left system, and April 3 in the right system as depicted in Fig. 3. This indicates an initial large-scale numerical adjustment of the post-emergence populations. This pattern of out-migration is supported by the population estimates plotted in Fig. 4. The resident populations stopped increasing following the main wave of emergence. The April 2 and April 10 estimates in the right channel are considered to be poor ones.

TABLE III. Numbers of coho and their position in the stream channels, March 13 to June 27, 1963.

	March				April				May		June
	13	15	19	26	3	11	18	28	18	31	27
Left channel											
Riffle 1	0	0	3	19	16	5	13	14	11	6	5
2	0	0	1	11	8	2	7	9	6	5	3
3	0	0	0	4	9	3	6	6	4	3	2
Pool 1		10	27	60	75	70	76	70	55	31	20
2	15	14	40	40	25	41	40	28	23	11	3
3	0	0	1	20	20	25	15	11	12	3	3
Total	15	24	72	154	153	146	137	138	111	59	36
Right channel											
Riffle 1	0	4	39	50	35	19	25	25	35	20	16
2	0	2	3	15	21	13	13	16	14	11	7
Pool 1	40	30	50	70	60	80	110	100	75	45	31
2	0	0	19	35	23	25	25	20	13	6	4
Total	40	36	111	170	139	137	173	161	137	82	58

As Table III indicates, a relatively fixed pattern of distribution persisted throughout the life of the experiment. The fry were concentrated toward the upstream ends of both channels. The extent to which this occurred and the accompanying degree of variability in numbers of fry occupying each environmental unit is depicted in Fig. 5. These plots are based on hourly fry counts in each environmental unit from sunrise to sunset.

This numerical gradient was associated with gradient in size as growth progressed. Due to the possibility of adversely affecting behavior, no extensive sampling of the two populations was made. However, the coho in the pools

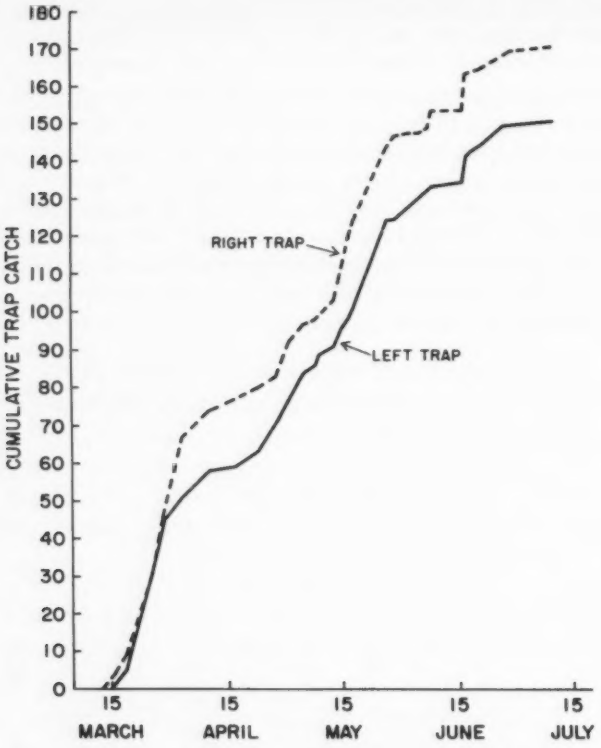

FIG. 3. Cumulative trap catch at outlet of each channel, March 13–July 15, 1963.

were sampled on May 1 and the resulting data are compiled in Table IV. In both channels the greatest average lengths were found in the first pools and were significantly different ($P = .05$) from those of the second pools. The mean length in pool 1 of the left channel was significantly greater than that of the third pool at the 10% level. The high mean length reported for the sample of eight fish taken from the latter pool is due to the influence of two large coho (50 and 53 mm). The remaining six fish fell within the range of 43–45 mm.

TABLE IV. Mean length in millimeters for coho sampled from the pools on May 1, 1963 and some related statistics.

	Left channel pools			Right channel pools	
	1	2	3	1	2
Sample size	39	17	8	52	16
Sample mean	48.03	43.76	46.25	48.02	45.63
Standard deviation	3.06	2.28	3.41	3.18	3.07

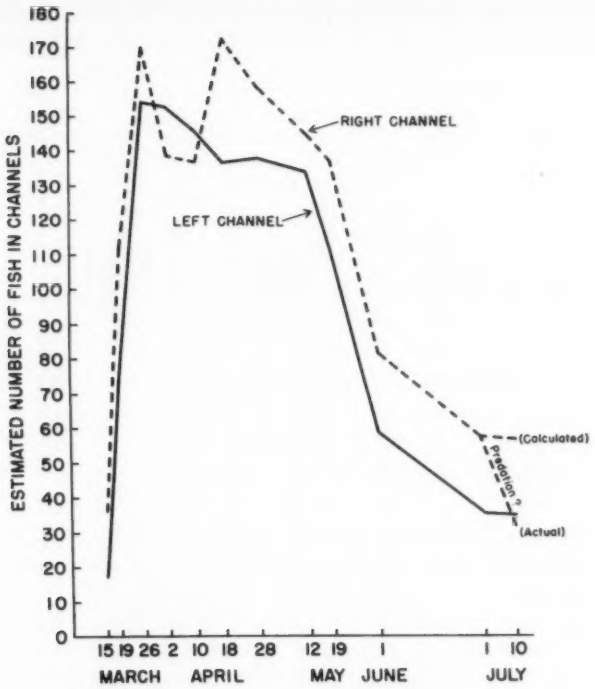

FIG. 4. Estimated number of coho in each channel, based on visual counts, March 15–July 10, 1963.

FATE OF FIRST-EMERGING COHO

The first coho to emerge from the gravel were marked by removal of the right ventral fin. The fate of these marked coho was followed in the trap catches, in the population samples, through behavioral observations, and by their contribution to the final populations upon termination of the experiment.

Marked fish emigrated in small numbers from both channels throughout the experiment as shown by Table V. In the majority of trap records pertaining to marked coho (12 of 14 cases) these fish exceeded the average length of the unmarked trapped fish. A sign test (Li, 1957) indicated that the difference was significant at the 10% level ($\chi^2 = 3.57$, d.f. = 1).

The average length was consistently higher in marked coho on May 1 in the right channel, and in both channels upon termination of the experiment (Table VI). The difference between the average lengths of fin-clipped and unmarked fish was amplified as growth progressed and was significant at the 5% level.

Marked coho were seldom observed in the second and third pools in the left channel and none was seen in the second (last) pool in the right system although

TABLE V. The average fork lengths of marked and unmarked coho in the trap catches.

Date	Channel	Unmarked		Marked		Sign of difference
		Number of coho	Mean length (*mm*)	Number of coho	Mean length (*mm*)	
March 15	R	1	38.0	2	38.0	0
" 19	L	4	38.0	1	39.0	+
" 26	L	23	38.9	3	40.6	+
" 29	R	14	40.0	1	42.0	+
April 6	L	2	42.0	1	47.0	+
" 18	L	1	43.0	1	46.0	+
" 28	L	6	42.5	1	47.0	+
May 5	L	6	47.5	1	47.0	-
	R	2	49.0	1	52.0	+
" 27	L	23	62.3	1	66.0	+
	R	19	54.9	2	64.3	+
June 8	R	4	65.0	1	69.0	+
" 17	L	6	60.6	2	79.0	+
	R	6	67.7	4	65.8	-
July 9	R	1	68.0	2	87.0	+

emigrants obviously passed these pools in order to enter the trap. Marked coho distribution correlates with the general size gradient in horizontal distribution previously discussed.

The experiment was terminated on July 10 due to an unidentifiable disturbance of the right channel population during the period between June 30 and July 1. A routine check on July 2 revealed that 16 fish had entered the trap and 16 were concentrated in the last pool. Twenty-five coho were missing. No fish were found upstream from the last pool and none moved back upstream

TABLE VI. Difference between average lengths of marked and unmarked coho on May 1 and July 10, 1963 (mm).

	Left channel		Right channel	
	Marked coho	Unmarked coho	Marked coho	Unmarked coho
May 1				
Sample size	Only one marked coho was taken in this sample		12	34
Sample mean			50.67	47.09
Standard deviation			3.75	2.58
July 10				
Sample size	5	30	8	24
Sample mean	84.25	76.48	84.86	77.54
Standard deviation	2.22	8.34	8.11	6.94

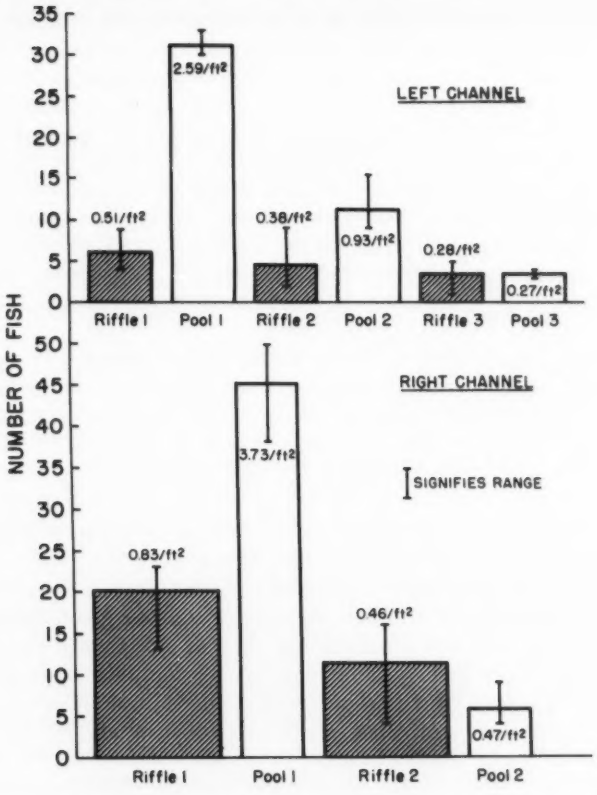

FIG. 5. Mean density of coho observed in each environmental unit, March 13–June 27, 1963, together with range in counts.

during the following 4 days although the trapped fish were released into the last pool. Three fish entered the trap during this time. During the next 4 days the general behavior of the population remained quite abnormal, the fish being highly sensitive to stimulation. Very low numbers of coho occupied the riffles.

There was no sign of interference in the left system and all fish were accounted for upon termination. Although the left channel was unaffected, avian predation in the right system was suspected. The final population means are given in Table VII.

Due to the loss in the right system, the number that would have been present was calculated by subtracting the accumulated trap catch since the last population estimate (June 27) from that estimate: 58 – 1 = 57 coho; in the left channel the number was 36 – 1 = 35 coho. The revised biomass values were calculated using both mean weights in case mortality in the right channel was exerted primarily on the smaller fish. Less than a 20-g difference resulted.

TABLE VII. Final population statistics, July 10, 1963.

	Left channel	Right channel	
		Actual	Revised
Number	35	32	57
Biomass (g)	166.3	160.3	270.8[a]–290.1[b]
Mean length (*mm*)			
All fish	77.59	79.32	
RV fish	84.25	84.86	
Mean weight (g)			
All fish	4.75	5.09	
RV fish	6.36	6.67	

[a]Based on mean weight of left channel coho.
[b]Based on mean weight of right channel coho remaining.

The percentage of marked coho increased from 15.3% to 25.0% in the right system, and from 8.3% to 14.3% in the left system.

A total of 212 and 234 fish, respectively, in the left and right channels, can be reliably accounted for out of 300 fish in each channel. Knowledge of the fate of remaining coho was not obtained due to trap inefficiency, cannibalism, or avian predation. Two dead fry were found in each container upon completion of emergence. Undetected emigration during the March–early April freshet period, avian predation and, less probably, cannibalism among the fry, are the most reasonable major sources of loss.

Data in Table VII indicate that there was little difference in size of fish in the two channels on July 10, 1963, but biomass in the right channel was greater. This suggested that the right stream channel was receiving a greater share of the organic drift due to the hydraulic conditions in the incoming water supply system. This hypothesis was tested by sampling the drift entering both channels with plankton nets. The result of a 24-hr series of samples taken every 3 hr with $\frac{1}{2}$-hr intervals (seven pairs of samples) revealed this to be the case. The right system received 36.7% more total settled volume of drift material and 36.4% more volume in potential fish food organisms. Available food level may be a primary factor among those determining the holding-rearing capacity of these artificial environments. In view of the distribution pattern of coho in the channels, and the associated behavioral aspects involving competition, this finding should not be surprising.

DISCUSSION

The coho fry first to emerge from the gravel enjoyed an ecological advantage over the fry emerging subsequently. The first-emerging fry were larger on the average than later-emerging fish, were relatively more abundant within the

terminal populations, and occupied the most-upstream areas within the stream channels.

Several factors could be involved in producing such an ecological advantage. Genetic superiority in such terms as constitutional vitality, greater growth potential, and above-average aggression levels may well be operating. It is doubtful that an ecological advantage was enjoyed by a portion of the embryos during development since incubation proceeded at oxygen saturation levels and normal temperature. The eggs were of above-average size and produced large and vigorous sac fry which spent 2–3 weeks in the gravel prior to emergence. Certainly any genetically endowed superiority would be difficult to separate from its ecological counterparts within the framework of the experimental design.

Assuming no important relationship between state of health and time of emergence from the gravel, there arises the distinct possibility that first-emerging fry have "settler's rights" to the available environment and/or the feeding opportunity to create or maintain a size gap between themselves and the later fry. Very small differences in size were found by Chapman (1962) to determine the outcome of aggressive interaction between coho fry, with the larger fish gaining social dominance. Furthermore, level of aggression was found to be higher among groups of coho of uniform size than among groups of disparate size. Similar observations were reported by Newman (1956) for mixed and homogeneous groups of rainbow and brook trout. It is postulated that social stability is positively related to growth depensation in single age-class populations and that size structure promotes social stability in multi-age-class populations. Ecological advantage in these juvenile coho is expressed through the medium of behavioral dominance dictating feeding opportunity for subordinate coho of lesser size. Other factors such as production of adrenocorticotropin due to socially instigated physiological "stress" as reported in brown trout by Brown (1957) may well be involved. Coho fry are cannibalistic and this propensity in age-0 smallmouth bass (*Micropterus dolomieui*) was reported by Langlois (1936) to result in a high degree of growth depensation.

The numerical and size distributions of coho throughout the stream channels are almost certainly associated with feeding opportunity and food availability levels, competition, and behavioral dominance through aggressive behavior. The populations depended almost entirely on the organic drift carried by the incoming water supply. This was supplemented to a small degree by surface feeding on ovipositing adult insects and the minor contribution made by insect production within the systems.

A significant positive correlation between level of aggression and water temperature may be an indirect result of increased locomotor activity and demands for food, cover, and space. Probably increases in temperature do not directly cause increased aggressive activity.

A positive correlation between level of aggression and rate of emigration from the stream channels would suggest that population adjustments in these systems are mediated by intensity of aggressive behavior. Factors encompassing

the food–space–shelter complex are no doubt of primary importance in population adjustments and related to aggressive behavior in juvenile coho. The role of aggression in hierarchical and territorial behavior has received much attention as a mechanism by which food and space are distributed within fish populations (Allee *et al.*, 1948; Collias, 1944; Hartman, 1963; Kalleberg, 1958; Kawanabe, 1958; Magnuson, 1962; Newman, 1956; Noble and Borne, 1938; Yamagishi, 1962).

Increased aggression following the introduction of food has been reported for several salmonid species including brown trout (*Salmo trutta*) and Atlantic salmon (*S. salar*) (Kalleberg, 1958); *S. salar* (Keenleyside and Yamamoto, 1962); rainbow trout (*S. gairdneri*) and brook trout (*Salvelinus fontinalis*) (Newman, 1956); Dolly Varden (*Salvelinus malma*), cutthroat trout, (*Salmo clarki*), and coho (Newman, MS, 1960). During late evening oviposition activity by adult aquatic insects at the water surface, aggression level in feeding coho was found to more than triple in the stream channels. Similar late evening rise in aggression and feeding activity was observed by Newman (1956) in rainbow and brook trout in Sagehen Creek.

It has been postulated that non-reproductive territoriality has been developed in stream-dwelling salmonids as a mechanism to assure adequate food distribution (Kalleberg, 1958). In coho, conventional competition for space has been substituted for direct competition for food, but this substitution is by no means complete. Direct competition involving both surface and subsurface feeding of coho was commonly observed in the pools and may be chiefly associated with a flexible hierarchical organization.

We postulate that social stability is an outcome of spatial fixation, these factors varying in positive relation to one another. The spatial fixation shown by coho supports the hypothesis that social stability is an important result of aggression in fish populations exhibiting hierarchical and/or territorial organization. The larger individuals dominate within the stream system. Strong fixation to territories was reported by Kalleberg (1958) for juvenile Atlantic salmon.

All available evidence indicates that the right channel yielded considerably more coho than did the left system, both in terms of numbers (63%) and biomass (78%). Random influences do not seem to have played an important role since the population trends are quite similar in both systems. We suspect that environmental circumstances of a consistent nature are involved in creating the dissimilarity.

A study of production of four year-classes of coho in three small streams in Oregon by Chapman (1964) indicated that production was more strongly correlated with the available stream area than with alternative environmental dimensions. If the carrying capacity of the environment is determined primarily by the spatial demands of the fish, the left channel should have held the most coho. It contained 50% more pool area and 20% more volume than did the right one. Therefore, spatial demands *per se* cannot be invoked in explanation.

The precise relationships between spatial requirements, aggression, and food availability level are yet to be worked out. Our results conflict with those of Chapman (1962) who reported that artificially high food level had no effect on the holding capacity of small, stream aquaria. The possibility should not be overlooked that short-term, high levels of feeding are not effective in stimulating juvenile coho to remain in an artificial system, whereas a sustained, elevated food availability which better simulates the natural drift might have a quite different effect.

The question of what constitutes "environmental capacity to produce coho" is not a simple one. It is closely associated with the problem of interpreting volitional residence in the present experiment. Some rather large coho left the systems, and the marked emigrants were generally larger than the average coho entering the traps. Other factors appear to be important in determining residency besides size, aggressive behavior, and their relation to food availability. It was suggested by Chapman (1962) that one possible explanation for "nomadic" coho larger than many resident fish is an insufficiency of suitable living areas for larger fish in the natural environment. These results emphasize that the ecological interpretation of behavioral studies, wherein volitional residence is denied, demands considerable caution. Further studies are presently underway to help clarify some of the above-mentioned problems.

ACKNOWLEDGMENTS

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A Solution of the Catch Equation[1]

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ABSTRACT

A method is described for explicit solution of the catch equation yielding year-by-year estimates of the population at the beginning of the fishing season and the coefficient of fishing mortality of fully recruited members of the population. A method of obtaining similar statistics for less than fully recruited ages is also given. Other types of information that can be derived if fishing effort is measured are discussed. A minimum program for solution of a fishery is described. As an example, the method is applied to the catch statistics of the Antarctic fin whale population.

INTRODUCTION

The catch from a population of fish during a unit time period is simply the population existing at the beginning of the time period multiplied by the fraction of the deaths ascribable to the fishery and by the fraction of total deaths.

$$(1)\quad C_i = N_i \frac{F_i}{F_i + M}(1 - e^{-F_i - M}) \qquad \text{where:}$$

C_i = the catch in the i^{th} time period;

N_i = the population at the beginning of the i^{th} time period;

F_i = the coefficient of fishing mortality during the i^{th} time period;

M = the coefficient of natural mortality (considered a constant unless otherwise specified).

The important assumption in the application of equation (1) is that all deaths in the population are accounted for, i.e. that the catches under study are all of the catches from the population.

This equation is basic in most approaches to (solving) fish population dynamics problems, e.g. the Beverton and Holt, and Schaefer methods (Schaefer and Beverton, 1963), though it has not been used directly because the information required for its solution is not available from the usual collection of statistics on landings and effort, the extreme being the absence of age data which led to the development of the Schaefer method. Because of this, second order properties of equation (1) usually have been utilized.

The most frequently employed of these second order properties is that the catch per unit of effort during a fishing season is proportional to the average population during the fishing season. This involves a number of important assumptions that are difficult to meet in practice. Among these are that the

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effort be measured without error and that the annual average effect of a unit of gear remain constant, i.e. that the behavior and distribution of the fish, and such factors as weather, remain constant from year to year. Paloheimo (1961) has explored some of the consequences of not meeting this assumption. Often these assumptions are met sufficiently closely to provide useable estimates of the maximum yield and the most efficient levels of fishing effort, but they frequently result in estimates quite inadequate for describing stock–recruitment relations where precise year-by-year estimates of stock and recruitment are essential — the more so if changes in the environment are involved in that relationship.

It is the purpose of this paper to describe an explicit solution of equation (1), a solution that involves two key assumptions, namely that the natural mortality coefficient is constant, and that the catches, accurately aged, are all of the removals from the population under study other than natural mortality, although as will be shown later, some relaxation of the second assumption is permissible under some circumstances. All that is required for the solution of a series of catches aged by time period is two extra pieces of information, e.g., an estimate of the natural mortality rate and an independent estimate of the fishing rate for 1 year.

The stimulus for the study was the critical need to describe more precisely the stock–recruitment relation of the Pacific sardine. The starting point was Widrig's (1954) pioneering attempt to deal with the varying availability of a given stock to a fishery. The results, however, proved to be of a more general nature than expected, so consolidated equations will be considered before returning to Widrig's concepts.

SOLUTION OF THE CATCH EQUATION

Let us first consider the catches from two successive seasons of a cohort of fish, and let us consider only the year-classes that are fully recruited into the fishery. The catch during one season is represented by equation (1). The catch in the following season is represented in equation (2):

$$(2)\quad C_{i+1} = N_i e^{-M-F_i} \frac{F_{i+1}}{F_{i+1}+M} (1 - e^{-F_{i+1}-M}),$$

where $N_i e^{-M-F_i}$ is the survival portion of the cohort (i.e. N_{i-1}).
Dividing equation (2) by equation (1) yields:

$$(3)\quad \frac{C_{i+1}}{C_i} = e^{-M-F_i} \frac{F_{i+1}}{F_{i+1}+M} \frac{F_{i+M}}{F_i} \frac{(1 - e^{-F_{i+1}-M})}{(1 - e^{-F_i-M})}$$

which is an equation with three unknowns, M, F_i, F_{i+1}. Let us now extend the series for a third year of catch data. Another equation (4) analogous to (3) may be written as follows:

$$(4)\quad \frac{C_{i+2}}{C_{i+1}} = e^{-M-F_{i+1}} \frac{F_{i+2}}{F_{i+2}+M} \frac{F_{i+1}+M}{F_{i+1}} \frac{(1 - e^{-F_{i+2}-M})}{(1 - e^{-F_{i+1}-M})}$$ in which

C_{i+1} now includes the next younger cohort. Equations (3) and (4) taken together are now a system of two equations, but the number of unknowns has been increased by only one, that is to four, by the introduction of F_{i+2}. The series can be expanded to j years (i.e. the total years for which catch data are available) yielding $j-1$ equations similar to type (3) and (4) and containing $j+1$ unknowns.

Hence, it is obvious that the system of equations can be solved explicitly yielding j solutions of F provided that two additional independent pieces of information are made available. These might be an estimate of the natural mortality coefficient (M) and an estimate of F for any year in the series. The latter might be derived from tagging experiments or survey-removal estimates (Chapman and Murphy, 1964). Fortunately, for many of the major fisheries of the world, e.g. the Pacific halibut or the Antarctic fin whales, such auxiliary information is available, at least in approximate form.

SOLUTION OF THE EQUATIONS

A system of equations of the type of (3) and (4) can only be solved by iteration. The approach can be more easily seen after collecting terms as follows:

$$Z = F + M$$

$$S = e^{-M-F} = \frac{N_{i+1}}{N_i}$$

$$a = 1 - e^{-F-M}$$

$$P^* = \frac{C_{i+1}}{C_i}.$$

Substituting and rearranging, equations (3) and (4) become (5) and (6):

$$(5) \quad P^*_i = S_i \frac{F_{i+1}}{F_i} \left(\frac{a}{Z}\right)_{i+1} \left(\frac{Z}{a}\right)_i$$

$$(6) \quad P^*_{i+1} = S_{i+1} \frac{F_{i+2}}{F_{i+1}} \left(\frac{a}{Z}\right)_{i+2} \left(\frac{Z}{a}\right)_{i+1}.$$

If we assume that M and F_i are known, equation (5) can be solved for F_{i+1}:

$$(7) \quad F_{i+1} = \frac{P^*_i}{S_i} F_i \left(\frac{Z}{a}\right)_{i+1} \left(\frac{a}{Z}\right)_i.$$

The penultimate term in (7) requires, of course, that the solution be iterated. Once F_{i+1} has been solved, F_{i+2} may be solved, and so forth until F_j (the last year of catch information) is solved. Back calculation from mid-series (say from F_{i+10}) which is also possible, is even more laborious, if not impossible by

hand, as a third non-linear term (S_i) is involved. In addition, the iterations must be repeated several times (say ten), because the solution of the series of non-linear equations is linked, resulting in rapid escalation of any arithmetic errors introduced by approximation. An alternate method, if the year with the known F is partway down the series, is to start with a trial value for F_i, compute down to the known F, and depending on the result, adjust the trial value of F_i until satisfactory agreement with the known F is achieved. A computer is virtually essential for either computational scheme because of the extreme requirement for convergence in the iterations.

Once the system of equations has been solved yielding the series of j solutions of F, the component parts of F can be examined. For example, if effort has been measured, F may be considered a product of (f) fishing effort and (q) the coefficient of fishing power, which are now all variables. Variation in q may be examined as a function of the behavior of the fish, weather, or as an approximation simply to indicate changes in the efficiency of the fleet. Under some circumstances it may be more efficient to initially divide F into its components. This approach will be considered next.

SOLUTION BY PRE-PARTITIONING F

As stated in the Introduction, the genesis of this study was the inadequacy of the average catch per unit of effort as a measure of population size of the Pacific sardine. This was the result of the variable availability of the population to the fleet from year to year. Widrig (1954) approached the problem by modeling a population as follows: during a given fishing season he divided the fishable stock into two components, one fully available to the fishery and a second not available at all. He further assumed that between fishing seasons there was complete mixing. This concept is expressed in equation (8), which is a modification of Widrig's (2):

(8) $\frac{N_{i+1}}{N_i} = S = S_a r_i + S_u (1-r_i)$ where the new terms are defined as follows:

S = survival rate;

r = fraction of the population available;

a,u = subscripts referring to the available and unavailable portions of the population.

Equation (8) can be expanded by appropriate substitution to:

(9) $\frac{N_{i+1}}{N_i} = e^{-F_i - M} = r_i e^{-M - vf_i} + (1-r_i) e^{-M}$ where v is an arbitrary constant modifying f. This model proved to be difficult to manipulate leading me to consider the simpler model expressed in (10):

(10) $\frac{N_{i+1}}{N_i} = e^{-M-(r_i v f)_i} = S_i$ where q, the coefficient of fishing power = rv.

The conceptual difference between equation (10) and (9) is primarily that equation (10) requires that all of the population be equally available to the fishery during a given season, i.e. the mixing process is continuous within and between seasons, but as in Widrig's model the average availability (r) varies among seasons. In effect v is now a residual constant and r a variable modifying vf. It can be seen at once that Widrig's model (9) might be more appropriate if the fishing season is very short, and equation (10) if the fishing season is long.

Whatever the philosophical difference, there is little difference in the numerical results, for taking logarithms and rearranging (9):

$$Z = M - ln\,[re^{-vf} + (1-r)]$$

$$\cong M - ln\,[1-r\,(1-e^{-vf})]$$

$$\cong M + r\,(1-e^{-vf})$$

$\cong M + r\,(+\,vf)$ where the approximations are the first terms in Taylor series expansion. Hence $Z \cong M + rvf$ which is equation (10) in log form. Empirical evaluation of (9) and (10) using values appropriate to a fairly heavy fishery confirms the near equality.

Equation (1) can now be rewritten as follows:

(11) $C_i = N_i \frac{r_i v f_i}{r_i v f_{i+M}} (1-e^{-r_i v f_i - M})$ where

v = a residual constant of proportionality of f;

f = fishing effort;

r = a variable reflecting changes in the effectiveness of the gear due to weather, etc., and/or errors in measuring f;

rvf = F, the fishing mortality coefficient as in (11);

rv = q, the catchability coefficient. (This notation preserves the classical meaning of q.)

Again, considering a series of j years of data, the equation analogous to (11) for the following year is:

(12) $C_{i+1} = N_i e^{-M-r_i v f_i - M} \frac{r_{i+1} v f_{i+1}}{r_{i+1} + v f_{i+1} + M} (1-e^{-r_{i+1} v f_{i+1} - M})$.

Dividing both sides of (11) and (12) by their respective f's, collecting terms, and dividing equation (12) by (11), yields:

$$(13)\quad \frac{C_{i+1}/f_{i+1}}{C_i/f_i} = S^* = S_i \frac{vr_{i+1}}{vr_i}\left(\frac{a}{Z}\right)_{i+1}\left(\frac{Z}{a}\right)_i$$

which is exactly analogous to equation (6). S^* in (13) is the usual catch per unit of effort in year $i+1$ divided by the catch per unit of effort in year i, whereas P^* in (6) is simply the quotient of the catches. For purposes of evaluation, equation (13) can be simplified and rearranged in the form of (7):

$$(14)\quad r_{i+1} = \frac{S^*_i}{S_i} r_i \left(\frac{Z}{a}\right)_{i+1}\left(\frac{a}{Z}\right)_i$$

and the pattern of solution is essentially similar to that of (7). The algebraic identity of the two methods may be appreciated by comparing equations (6) and (13). If, when using the second method, one has a series in which effort has not been measured, and one simply inserts the same arbitrary large number for each f, the two methods converge in that they are both free of the necessity of measuring f in order to evaluate F.

NOTES ON COMPUTATION

Although this study began on the basis of pre-partitioning F into *rvf*, it is obvious that often it will be easier to employ the consolidated solution, and subsequently examine F for its components. (I have a working program for the pre-partitioning method that can be furnished on request.)

The second choice to be made is whether to start with the known value of F (assuming M is the second piece of information) and compute in both directions, or to start at year 1 and iterate to the known value until satisfactory agreement is obtained. If the known value of F is absolutely precise either way will do. If it is not precise, and if it is, say, midway in a 20-year series, the values at the end of the series will usually be obviously ridiculous because of the linked nature of the system of non-linear equations.

On the other hand, computation from the first year (forward computation) using a systematically incremented spectrum of values for F_i will result in a spectrum of solutions. Then all of the extra information on the fishery can be brought to bear in selecting a most probable solution. If the $n+1$ degree of freedom is an estimate of average F over 2 or more years, say from tagging, it is obvious that this information can be brought to bear only as a criterion of selection, thus requiring that the forward method be used. If there are several independent estimates of F in a series, one might select the solution that minimized the variance between the computed and estimated F's.

Essentially the same remarks apply to the second, or pre-partitioning method of solution except that a series of solutions of r, associated with systematic increment of v is the result. In the particular program I have developed, the

convention has been adopted of designating the r for the year with the maximum coefficient of F, ($rv = q$), as equal to 1.0. This has the advantage of keeping the numerical results within the usual working spectrum of values, and, if r can be interpreted as a function of the behavior and distribution of the fish, there is some logic and considerable convenience in designating the "best" year as unity.

Finally, most of this study was based on a set of artificial data, 20 "years" in length, constructed to resemble the California sardine record, relying on random tables for the selection of r's and f's, and year-class sizes. This set for which all true values were known served as a program and algebra check. Such a set is highly recommended when solving a real problem as it can be used to examine the effect of suspected real errors on the solution, e.g. mis-estimation of M.

ERRORS IN THE TWO PRIMARY ASSUMPTIONS

As stated in the Introduction, there are only two primary assumptions involved in the solution of the catch equation. Secondary assumptions are, of course, involved in the interpretation of the r values recovered from the pre-partitioning solution (or after partitioning F if the general solution is used). For instance, if r is to be interpreted as having meaning with respect to the behavior and distribution of the fish, f must be assumed to be measured without error. Or, if it is desired to assume that r represents changes in gear efficiency it must be assumed that the availability of the population remained constant. Such problems are secondary to the assumptions that natural mortality is constant and that the catches under consideration are all of, and only from, a unit population.

Natural Mortality Assumption

At best, one starts with a single average value of M for the series. It is not likely that this value will be real for any single year. Such errors, of course, decrease in importance as F becomes large, not as a function of the per cent error in M or the relation of M to F, but rather in proportion to the *error in* M relative to the entirety of F. In any heavily exploited population this must be small. Similar considerations apply to systematic error in the estimate of M.

The Catch Assumption

The ideal situation is one involving a unit population with no emigration or immigration, and with adequate age sampling from all catches. Situations short of this ideal may still yield useful solutions. For example, if the catches from only a portion of the population are aged but the total catch is known, and if it can be assumed that the catches from the unsampled area have the same age composition as the sampled area, satisfactory solutions result. Obviously, however, the basic equations (1) and (5) assume that all mortality is accounted for. Therefore, any unaccounted for mortality or migration will introduce

errors into the solution, and owing to the linked calculation an error during just one year will affect all other years.

APPROXIMATE AND INDIRECT SOLUTIONS

If the appropriate extra degrees of freedom are not available in the stricter sense, recourse may be had to other properties. Perhaps the least obvious of these stems from the linked-non-linear nature of the set of equations. If, say, there is a reasonable guess for the value of the natural mortality coefficient but no independent estimate of F, the second degree of freedom can simply be a "reasonable" appearing solution, i.e. one in which none of the F's is too high or too low. Such a solution may not be appropriate for recovering the population size N, but it will usually give a good estimate of the relative magnitude of F or r from one year to the next.

Other properties that may be useful as a source of a second degree of freedom relate to the pre-partitioning solution. For example, if it can be assumed that fishing effort f is measured without error, it might be assumed that r reflects year-to-year changes in the availability of the fish. If it can be further assumed that these changes are drawn from a symmetrical distribution, one can select the solution that minimizes the third moment, or symmetry parameter. In the artificial data I have studied, the r's of which were drawn from a random table, this criterion resulted in the selection of a solution with a maximum deviation of 5% from the true values. Other information about r might also be used. For example, if during 1 year the vessels were placed on half-capacity limits owing to economic factors, the relation between that r and adjacent r's could be prescribed *a priori* and used to select the "best" solution.

Another possibility for an approximate solution can be derived from:

$$(15)\quad \sum_{i}^{j-1} Z^* = -ln\, r_i + ln\, r_j - ln\left(\frac{Z}{a}\right)_i + ln\left(\frac{Z}{a}\right)_j - \sum_{i}^{j-1} (M + rvf)$$

which is the summation of the j–1 equations of type (13) in log form. Of the five terms on the right hand side, the first four are correction terms so that if the series is long, e.g. $j = 20$, and an estimate of M is at hand, an approximate solution for rq can be obtained by ignoring the first four terms. This can then be used as a second degree of freedom in selecting an appropriate solution.

SOLVING FOR YOUNGER AGE CLASSES

The calculations already described deal only with the older classes or cohorts which are presumed to share the same values of M and F. Once the value of F is computed for a given year, the population N of each of these age classes at the beginning of the fishing season may be solved for in equation (1). Often, however, it is desirable to solve for the population of the younger cohorts or age groups. The population sizes of these might serve, for example, as estimates of year-class size. The problem can be solved readily as follows:

Let:

N_0 = size of the year-class not fully recruited.

C_0 = catch of the year-class not fully recruited.

F_0 = fishing rate on the year-class not fully recruited.

N_1 = size of the year-class 1 year later when it is fully recruited. (This will have already been estimated from the above scheme.)

Then:

$$(16) \quad N_1 = N_0 e^{-F_0 - M}$$

$$(17) \quad C_0 = N_0 \frac{F_0}{F_0 + M} (1 - e^{-F_0 - M})$$

which together form a system of two simultaneous equations in two unknowns, F_0 and N_0. They can be solved for N_0 by iteration of F_0. Obviously this procedure can be extended backwards to younger cohorts so long as the catch of these is substantial.

MINIMUM INFORMATION TO STUDY AN EXPLOITED POPULATION

The analytical framework described in this paper can be used to specify a minimum research program for measuring a population of fish and measuring the response of that population to exploitation, as follows:

1. Definition of the stock;
2. Complete account of the catch from that stock;
3. Adequate representation of the age structure of the catch;
4. An estimate of the natural mortality rate of the stock and an estimate of the fishing rate (F) for 1 year, or independent estimates of the fishing rate for 2 years. In a continuing important fishery it would be well to independently estimate F periodically, say every 5–10 years.

This scheme leaves out the classical need to measure fishing effort (f). Very obviously, if a complete program (not minimal) is desired, f should also be measured, because the equations can then provide a great deal more information ranging from insight into the biology of the stock, to objective economic evaluation of innovations thought to increase the efficiency of the fleet. An example of the latter might be the selection of the most efficient size of fishing vessel.

AN EXAMPLE

Though this work was done with an analysis of the Pacific sardine in view, that particular problem is too frought with secondary complexities to serve as a straightforward example. Rather than go to the other extreme and use the set of artificial data, statistics for the Antarctic fin whale population provided by D. G. Chapman are analyzed. The basic data and the results, using the pre-

TABLE I. Statistics on Antarctic fin whale fishery, all areas. January, February, March catches.

All statistics refer to males only.
E = fraction of N taken by the fishery, i.e. $E = \frac{F}{F+M}(1-e^{-F-M})$
M = .05
Selection criterion was $\overline{F}$ 1954–1958 = 0.29 from tagging experiments.

	Ages 4 and up							Age 3 only			Age 2 only		
Year	f[a]	S*[a]	$rv = q_a \times 10$	F	E	C	N	F	C	N	F	C	N
1946	8,253	.678	.534	.044	.042	2723	64,708	.054	382	7519	.072	381	5605
1947	14,414	1.711	.401	.058	.055	4184	76,368	.120	547	4958	.097	494	5493
1948	17,747	.829	.796	.141	.129	7321	56,973	.200	850	4745	.132	675	5614
1949	18,902	.816	.803	.152	.137	6349	46,200	.169	712	4684	.120	612	5547
1950	18,128	.774	.799	.145	.132	6500	49,407	.165	693	4680	.130	675	5676
1951	18,952	1.230	.750	.142	.129	5812	44,942	.161	687	4742	.107	737	7444
1952	16,902	.645	1.146	.194	.172	8430	49,031	.158	909	6364	.145	807	6127
1953	17,101	1.542	.927	.159	.143	7193	50,269	.299	1271	5043	.194	1324	7711
1954	15,630	.600	1.878	.294	.248	9027	36,328	.270	1398	6046	.258	1531	6899
1955	16,619	1.049	1.564	.260	.223	8330	37,284	.392	1607	5071	.283	1726	7171
1956	14,893	.575	2.330	.347	.286	9517	33,225	.281	1231	5141	.194	1337	7783
1957	15,429	.564	1.949	.301	.254	9524	37,553	.184	1000	6100	.166	1060	7090
1958	16,344	.891	1.524	.249	.215	8288	38,499	.308	1477	5712	.242	1475	7041
1959	16,275	.535	1.882	.306	.258	8631	33,487	.359	1551	5261	.301	1555	6135
1960	21,269	.882	1.437	.306	.257	7956	30,941	.397	1384	4322	.306	1390	5398
1961	23,998	.449	1.949	.468	.365	8090	22,161	.499	1451	3780		1385	
1962	29,952		1.446	.433	.344	6201	18,049		1178			1386	

[a]These are the input data for the computer program; "f" is catcher boat days.

partitioning-of-F scheme of analysis, are given in Table I. The input data for the computer program were the f and S* values and the natural mortality coefficient. The last degree of freedom, which was the basis for selecting the appropriate solution, was an estimate of average F for the years 1954 and 1958 based on tag returns. The statistics used are on males only. Presumably these represent about half the population.

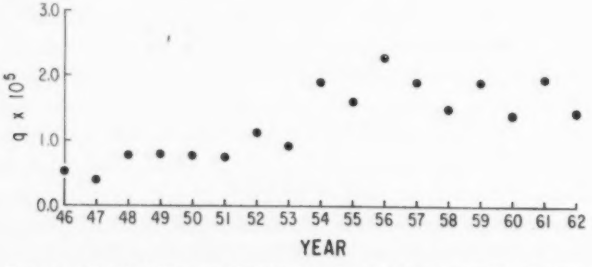

FIG. 1. Catchability coefficient (q) plotted as a function of time. The trend in q is probably a function of increased size of the catchers as well as technical innovation. The peak (1956) coincides with very heavy exploitation of a sanctuary zone opened in 1955.

As examples of the use of the results in Table I, Fig. 1 and 2 show the change in $rv = q$ with time, and the parental stock–recruitment relation. The general trend in q shown in Fig. 1 might be interpreted as an increase in the efficiency of the fleet, and the scatter as representing changes in fishing conditions from year to year, e.g. weather. The stock in Fig. 2 is the population of ages 4 and over and the recruitment is the appropriate estimate of the populations of age 2, the oldest not yet fully recruited age for which the catch was substantial. A full consideration of these results, together with comparisons with other analytical techniques is in press (Chapman *et al.*, 1964, 1965). For a general account of this fishery see Laws (1962).

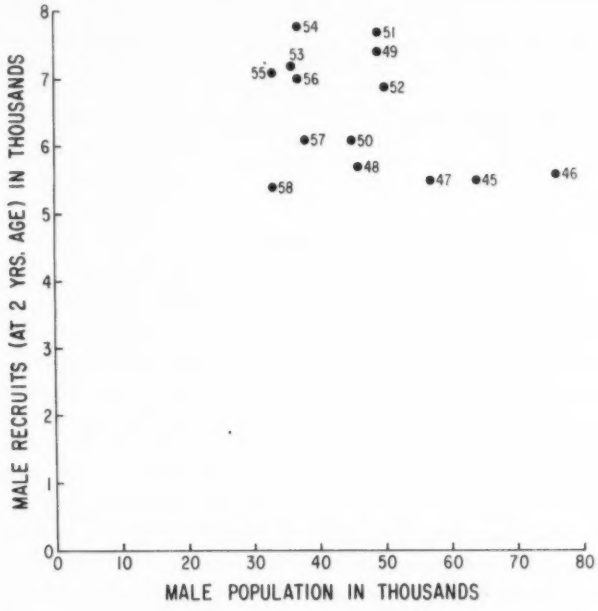

FIG. 2. Relation between the breeding population and numbers of young surviving to the beginning of the fishing season 2 years later at which time they are about 2 years old. Breeding stock is taken as the number of adults surviving to the beginning of the fishing season following the season of birth.

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Comparative Zone Electropherograms of Muscle Myogens and Blood Hemoglobins of Marine and Freshwater Vertebrates and Their Application to Biochemical Systematics[1]

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ABSTRACT

The muscle myogens of approximately 50 species of fishes, representing specimens from three (Elasmobranchii, Holocephali, Teleostomi) of the four living classes of fishes of the superclass, Gnathostomata, have been separated by starch gel zone electrophoresis. Evidence is presented for the virtual constancy and species specific nature of these myogen as well as their corresponding multiple hemoglobin patterns. The interspecific and intergeneric similarities of myogen patterns within any one family (Salmonidae as well as Scorpaenidae) were remarkably parallel with the existing morphometric classification. The same degree of relationship was not observed in the corresponding hemoglobin patterns. The usefulness of myogen, and less frequently, hemoglobin patterns for obtaining phylogenetic information as well as for species identification purposes is discussed.

INTRODUCTION

The application of separation and structural studies of proteins to taxonomic problems under the general topic of biochemical systematics (Alston and Turner, 1963; Manwell and Ann-Baker, 1963; Marmur *et al.*, 1963) is receiving increasing attention. It is now a well-established biochemical fact that genetic information coded into molecules of deoxyribonucleic acid (DNA) are translated through a series of reactions into the structure of proteins (Perutz, 1962; Yanofsky *et al.*, 1963; Nirenberg *et al.*, 1963; Ochoa, 1963; Crick, 1963). The analyses of proteins, therefore, are considered to be the simplest indirect approach to those problems (Marmur *et al.*, 1963). Sibley (1962) states: "Since protein molecules are the principal morphological units of the animal body at the molecular level of organization, it follows that their form and structure are as relevant as sources of genetic and phylogenetic information as are the muscles, bones, organs, skin, hair, feathers and other structures which themselves are composed largely or entirely of protein molecules."

Anfinsen (1959) was one of the first to appreciate the interrelationship between protein and gene structures and his views are described in his book, "The Molecular Basis of Evolution". It was this monogram that inspired Sibley (1960) to study over 5000 paper electrophoretic profiles of avian egg-white proteins for classification purposes. Other references to electrophoretic studies on blood proteins of mammals and birds are reviewed in the same article. Engle and Woods (1960) have reviewed the subject of plasma proteins prior to 1960. Over some period of time, Dessauer and Fox (1956) examined by paper

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electrophoresis, 1200 plasma patterns from more than 800 specimens of at least 100 species of amphibians and reptiles. Certain general diagnostic characteristics enabled the authors to assign these animals in their proper orders.

In marine and freshwater vertebrates and invertebrates, electrophoretic studies were carried out on the serum proteins of several species of sharks (Starr and Fosberg, 1957), 8 elasmobranchs, 12 teleosts (Engle *et al.*, 1958) and 19 species of invertebrates (Wood *et al.*, 1958). A more extensive study of plasma proteins of 14 families and 26 species from the Gulf of Mexico were carried out by Sulya *et al.*, (1961). Several investigators were able to demonstrate a decrease in complexity as well as total plasma proteins from higher to lower fishes (Gunter *et al.*, 1961; Manwell, 1963). Very little albumin (Manwell, 1963) was found in the cyclostomes, morphologically the most primitive of living vertebrates, while a condition of analbuminemia existed in elasmobranchs (Sulya *et al.*, 1961; Irisawa and Irisawa, 1954; Drihlon and Fine, 1959). The complexity of globulin patterns likewise showed a general decrease toward the lower forms (Gunter *et al.*, 1961).

Hemoglobin polymorphisms have been reported in a wide range of species of fishes (Schuman, 1959; Sick, 1961, 1962; Chandrasekhar, 1959; Buhler, 1959, 1963; Hashimoto and Matsuura, 1959a, b, c, 1960) although the extent was not fully appreciated until the highly sensitive starch-gel zone electrophoretic techniques were applied to their separations (Tsuyuki and Gadd, 1963; Manwell *et al.*, 1963). The higher forms of fishes displayed equal or greater complexity than mammals. Comparative differences were then emphasized and more useful phylogenetic information realized. In the primitive cyclostomes the hemoglobin patterns are characterized by simplicity (Manwell *et al.*, 1963) compared to higher forms, and structurally the molecule consists of a single polypeptide as indicated by its molecular weight of about 17,000 (Allison *et al.*, 1960; Roche and Fontaine, 1938, 1940) similar to vertebrate muscle myoglobin. In marked contrast, the hemoglobin from *Salmo gairdnerii* has a molecular weight of 68,000, while that from *Oncorhynchus keta*, 61,000, and 72,000 for the fast and slow components (Hashimoto and Matsuura, 1959a). This order of molecular weight is consistent with the four polypeptide structure of mammalian hemoglobins. However, there appears to be some doubt in the number of polypeptides present (Buhler, 1963) in the hemoglobin molecule from *S. gairdnerii* and *Oncorhynchus tshawytscha*. As the extent of polymorphism has been shown to be much greater in these species of fishes (Tsuyuki and Gadd, 1963), the molecular weight studies carried out on the basis of two or three components should be reinvestigated.

Further interesting results may be obtained from a comparison of peptide maps. The major hemoglobins of *Petromyzon marinus* and *Ichthyomyzon unicuspis* have identical electrophoretic mobilities (Manwell, 1963). In their overall patterns, *Petromyzon marinus* have two additional minor zones. However, the major hemoglobin fingerprints of the two species of lampreys differ only in the chromatographic migration of two peptides, indicating at least two mutational differences (Manwell, 1963). Another example of two hemoglobins with identical

mobilities but different fingerprints is found in certain strains of laboratory mice (Gluecksohn-Waelsh, 1960). The difference here is in only one neutral amino acid (Popp, 1962). These substitutions in neutral amino acids will be more important for the comparison of hemoglobins from closely related species such as the salmonids where a number of zones with identical mobility are found in most members of the family.

What appears to be more useful for obtaining phylogenetic information is a group of muscle proteins soluble in salt solutions of low ionic strength. The muscle myogen patterns show a high degree of species specificity when separated by starch-gel electrophoresis (Thompson, 1960; Giles, 1962; Tsuyuki *et al.*, 1962; Tsuyuki and Roberts, 1963). These observations are of particular value as further descriptive criteria in the identification of fishes. A remarkable degree of parallelism was found to exist between Berg's (1947) classification of fishes, particularly within families, and the similarity of muscle myogen patterns.

This communication describes the results of further studies carried out over the past 2 years on the myogen and hemoglobin patterns of representative samples from three (Elasmobranchii, Holocephali, Teleostomi) of the four living classes of fishes of the superclass Gnathostomata, and their relationship to the existing order of classification.

MATERIALS AND METHODS

Most of the specimens were collected from English Bay and Indian Arm (British Columbia) areas on board the *Investigator No. 1*, and from the Gulf of Alaska on the research vessel *G.B. Reed*. Freshwater samples were collected locally.

Immediately after hauling on board the vessel, each fish was wiped free of excessive water and bled from the caudal artery by severing its tail and the blood collected in 2-oz wide-mouth jars which had previously been rinsed out with a 10-mg/ml aqueous solution of heparin and dried. The blood samples were then stored on ice. The carcasses were put in polyethylene bags and frozen immediately in dry ice. In large fishes, a piece of flesh of about ½ lb was sliced from the mid-section and frozen.

Preparation of Hemoglobin and Muscle Myogens for Electrophoresis

Blood samples were centrifuged at 3000 rpm and the clear plasma removed. Red cells were washed three times with 1% sodium chloride solution, lysed with seven volumes of distilled water and stroma removed by centrifugation, (Tsuyuki and Gadd, 1963). The solution of hemoglobin prepared in this way within 3 hr after sampling was brought to the laboratory as quickly as possible and applied directly to the starch-gel slots for electrophoresis. Muscle myogens were prepared as described previously (Tsuyuki *et al.*, 1962).

Electrophoresis

Certain deviations from the standard starch-gel technique, employed in previous studies (Tsuyuki *et al.*, 1962; Tsuyuki and Roberts, 1963), have not

been fully reported and will be described in more detail at this time. Construction details and type of gel casting trays employed in these studies have been described fully elsewhere (Tsuyuki, 1963). For the separation of hemoglobins a tray of one-half the depth was employed. To minimize over-heating on the hot plate the starch suspension was stirred mechanically with a stirrer constructed to sweep the entire flat bottom surface of the suction flask. To overcome the viscosity of the starch-gel, a heavy duty stirring motor was found to be required. The outside walls of the flask were wrapped in aluminum foil to minimize cooling. To avoid excessive buckling of the tray, the entire gel casting tray assembly and the corresponding slot forming cover were heated in a large oven to 75°C before the hot gel was poured. All parts of the tray and cover which come in contact with the gel were lubricated lightly with mineral oil prior to heating. The whole tray, supported in the centre, was filled with hot gel and covered with the slot former. Three pieces of $\frac{1}{4}$-inch plywood were then placed on top and weighted evenly with six 10-lb blocks of lead.

The muscle myogens were separated in starch-gel prepared in 0.023*M* sodium borate buffer at pH 8.5. The salt reservoir consisted of 0.3*M* sodium borate of the same pH. For hemoglobin solutions, it was found that sharper resolutions were obtained when 0.1% EDTA was added to the gel buffer (Vanstone *et al.*, 1964). The pH in this case measured 8.0. All hemoglobin separations in these studies were carried out under these conditions.

After the gel was set and had cooled to 0°C in the refrigerator, the cover was removed carefully and the gel wrapped smoothly with Saran Wrap in a manner described previously (Tsuyuki, 1963). The entire tray was now placed on a 45-cm E-C electrophoretic unit so that the two end extensions of the gel were immersed in the bridge buffer solution. The electrophoresis was carried out horizontally and at 0°C. The sample was introduced in the slots which were then covered with strips of Saran Wrap. Muscle myogens were separated for 24 hr and the hemoglobins for 28 hr at a potential gradient of 250 across the length of the gel. When electrophoresis was completed, the wick ends of the gel were cut off. The thicker gel, which is $\frac{3}{8}$-inch in thickness, could be sliced into three equal pieces of $\frac{1}{8}$-inch and one of these was used for staining with amido black. The casting tray was used as the slicing board by inserting $\frac{1}{8}$-inch plexiglass dividers between the gel and tray. The $\frac{3}{16}$-inch hemoglobin gels were stained without slicing. Staining and washing procedures were carried out in $1\frac{1}{2} \times 4 \times 18$-inch stainless steel troughs which were gently shaken horizontally at the lowest speed of an Eberbach variable speed shaker.

The stained gels were photographed both by reflected and transmitted light by the photographic section of the Department of Extension at the University of British Columbia. To avoid excessive heating and subsequent drying of gels, electronic flash was later used as the source of transmitted light.

RESULTS AND DISCUSSION

To facilitate the presentation of results, fishes from which samples were obtained were arranged in the order of classification according to Berg (1947).

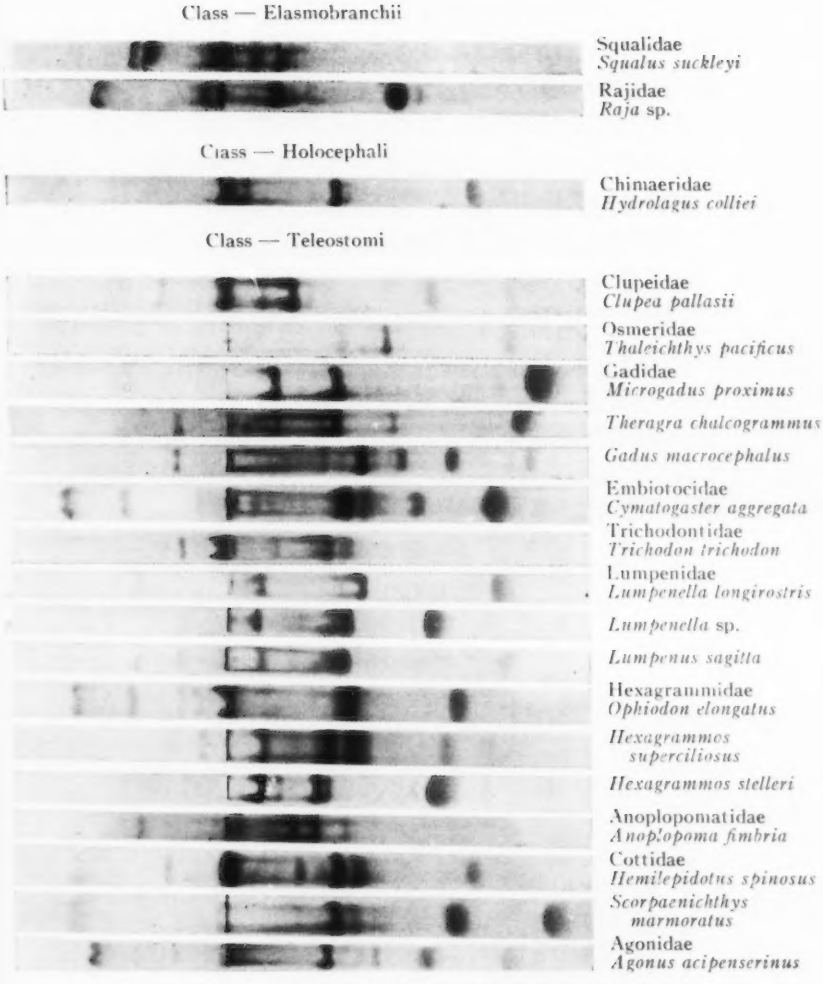

FIG. 1. Myogen patterns of fishes (cathode to left, anode to right).

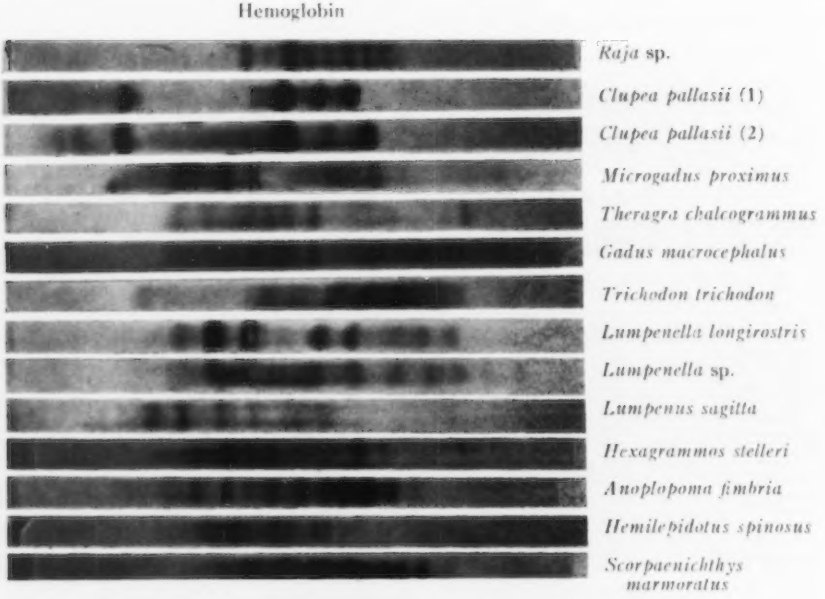

FIG. 2. Multiple hemoglobin patterns (cathode to left, anode to right).

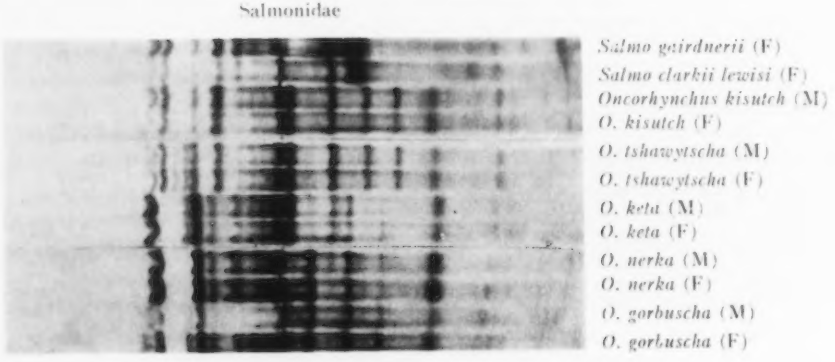

FIG. 3. A direct comparison of myogen patterns of some members of Salmonidae. Letters in parentheses indicate male (M) or female (F) (cathode to left, anode to right).

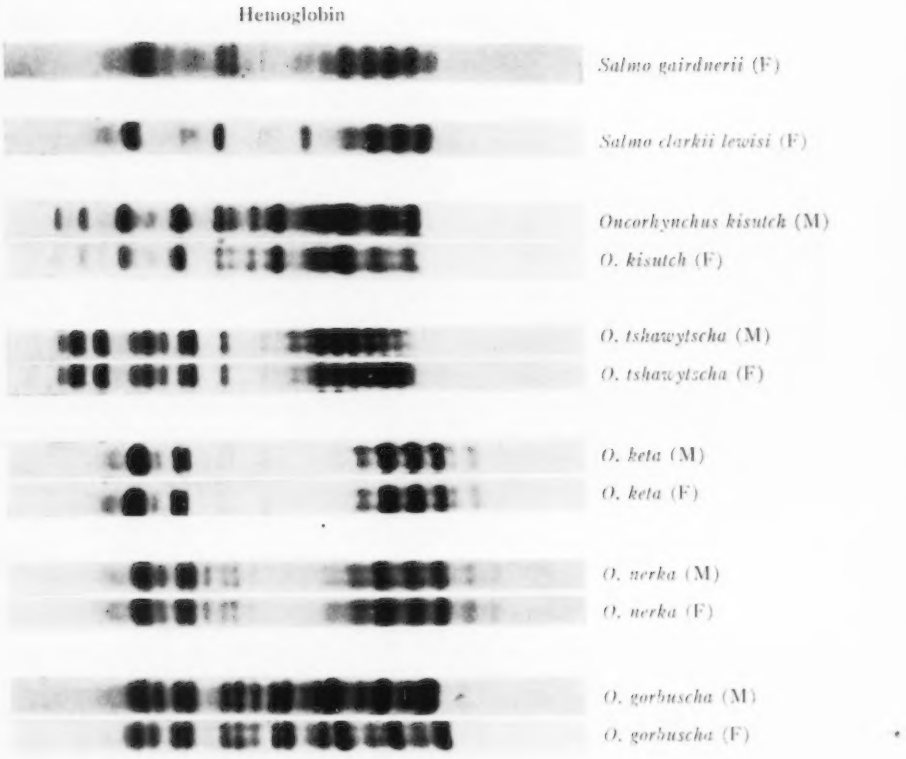

FIG. 4. Multiple hemoglobin patterns of some members of Salmonidae. Letters in parentheses as in Fig. 3. Comparative runs on the males and females of the genus *Oncorhynchus* were run simultaneously (cathode to left, anode to right).

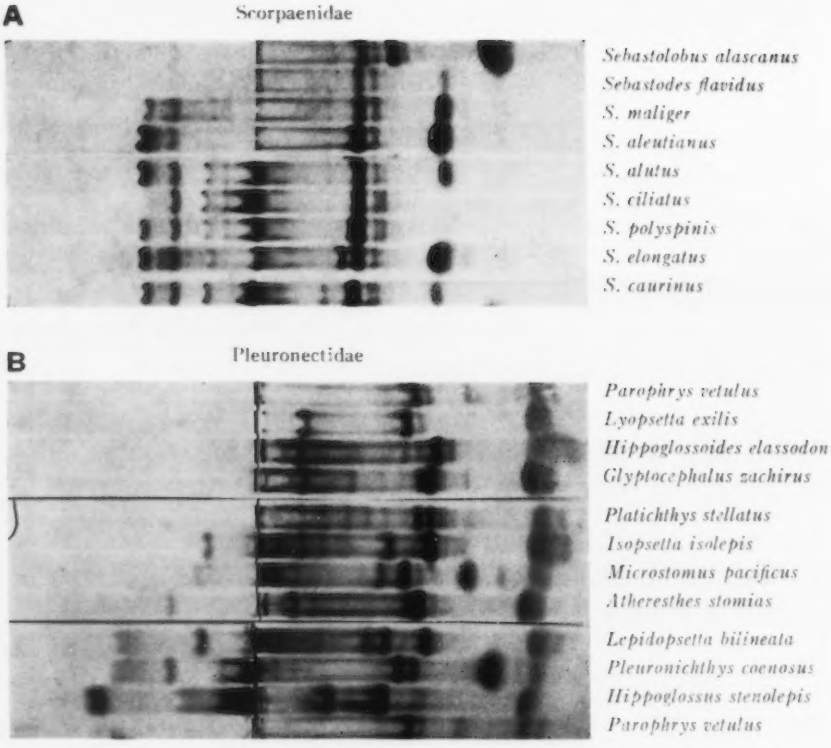

FIG. 5. A. A simultaneous run of the myogens of Scorpaenidae. B. A simultaneous run of the myogens of Pleuronectidae (cathode to left, anode to right).

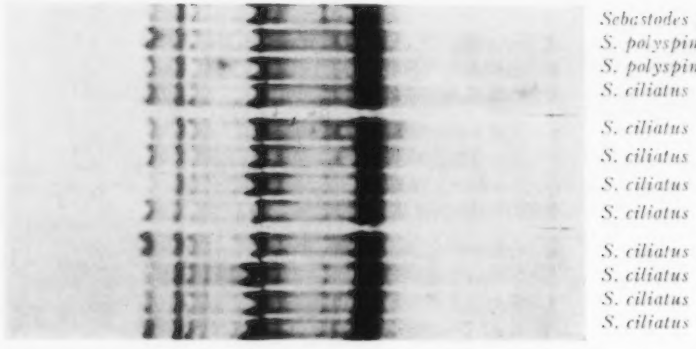

FIG. 6. Myogen pattern variations found in three specimens of *S. polyspinis* and nine specimens of *S. ciliatus* (cathode to left, anode to right).

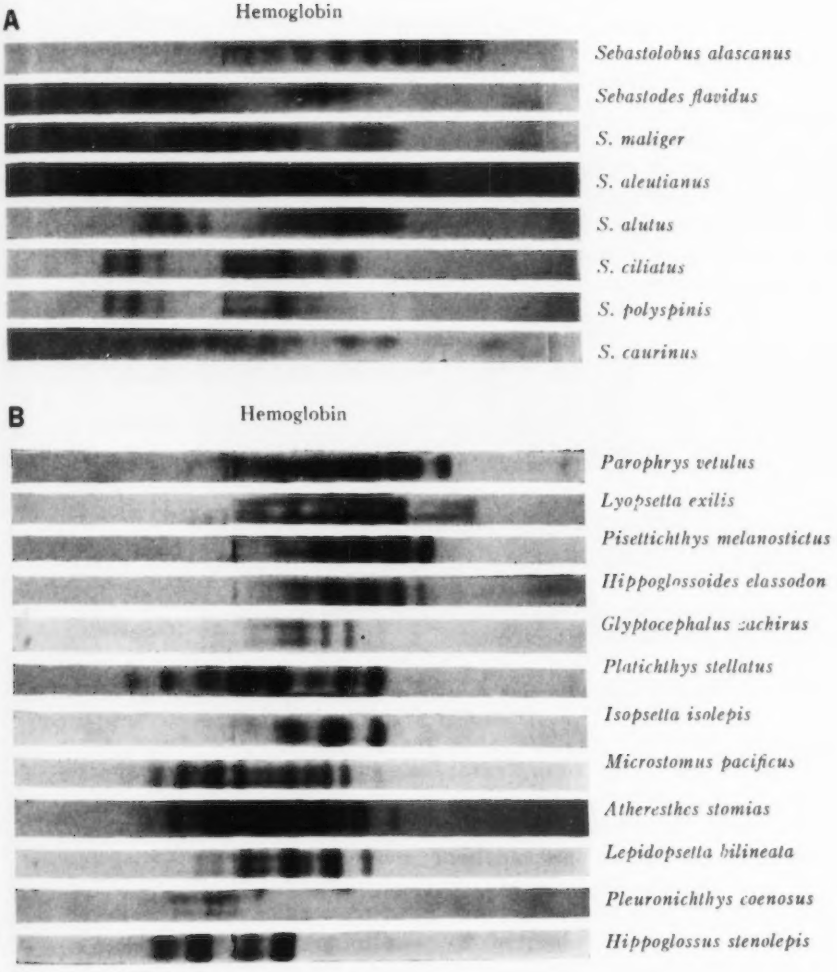

FIG. 7. A. Multiple hemoglobin patterns of Scorpaenidae. B. Multiple hemoglobin patterns of Pleuronectidae (cathode to left, anode to right).

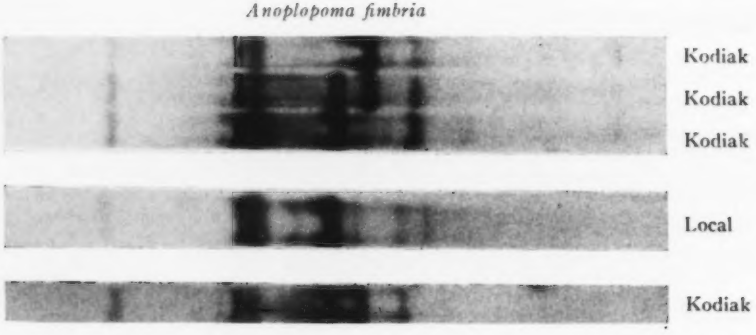

FIG. 8. Variations in myogen pattern of *Anoplopoma fimbria* captured in the Gulf of Alaska, and the Strait of Georgia (cathode to left, anode to right).

One pattern each of muscle myogens and hemoglobins is presented since an examination of large numbers of specimens of any one species showed that only on rare occasions did variations occur. Where such variations were obtained, those results are presented and discussed separately. Marine and anadromous species are remarkable in that their protein pattern characteristics are relatively constant, while the freshwater species from the Great Lakes area exhibit considerably more variations (private communication, Mr J. Uthe).

The myogen patterns of fishes are presented in Fig. 1 with the exception of the salmonids, rockfishes, and flounders which are discussed separately to emphasize intra-family relationships. It is quite apparent that no two myogen patterns are exactly alike in the 46 species examined. Conversely, it is possible to state that in these samples the myogen patterns are specific for any one species and constitute a single criterion by which these fishes may be identified.

The question of the effect of environmental conditions and the physiological state on these myogen patterns is, understandably, raised frequently. If the proteins produced in fishes follow basically the same biosynthetic route as in other living organisms, the biochemical reactions leading to their formation are subjected to the same factors which enable their chief genetic material, DNA, to lead to the synthesis of specific proteins according to the genetic requirements of that organism. This synthetic process should not be altered by environmental conditions if the continuity of a species is to be maintained, barring any drastic mutational changes.

A study of well over 650 individual sockeye salmon (Tsuyuki *et al* .,unpublished data) of both sexes from 27 spawning grounds, ranging from Alaska through British Columbia to the State of Washington, and at different stages of sexual maturation during their river migration, showed that their myogen patterns are virtually identical. Even more significantly, the myogens of underyearling salmonids in fresh water already display patterns readily identifiable with their adults.

The results of Fig. 1 do not show any correlation between complexity of patterns with increasing order as some investigators have found in the case of serum proteins. It is reasonable to suppose that any such molecular complexities would have occurred long before the time of the earliest living forms of fishes. Where more than one species of one family has been examined, certain generic relationships exist in the similarity in the electrophoretic mobilities of several of the protein zones. The main point demonstrable in Fig. 1 is the absolute species specificity and reproducibility of the protein patterns. In the family *Lumpenidae*, the pattern of the second *Lumpenella* species shown bears no species label since, unfortunately, no identification was available.

The hemoglobin patterns of many of the species investigated in the previous figure are shown in Fig. 2. Where patterns are not available, the blood samples were either badly hemolysed or were not possible to analyse before deterioration. In many of the species the standard conditions employed for obtaining whole blood resulted in extensive hemolysis. Samples of blood from the *Clupea pallasii* obtained at Porpoise Bay and Porteau in Howe Sound show slight variations.

The remainder of the samples display a degree of species specificity similar to their myogen patterns. The extreme hemoglobin polymorphism observed in the salmonids (Tsuyuki and Gadd, 1963) are again found in these species.

There are very few meristic characteristics which do not exhibit degrees of phenotypic variation induced by environmental factors (Rounsefell, 1962). For instance, the rearing temperature influences the formation of vertebrae in young *O. tshawytscha*. Other characteristics as branchiostegal rays, pyloric caeca, fin rays, gill rakers, and scales, though useful for quick examinations, show sufficient interspecific overlap as well as range to necessitate large sampling. There is no one factor that can be relied upon to distinguish all members of the Salmonidae. In contrast, the muscle myogen (Fig. 3) and hemoglobin (Fig. 4) patterns of the salmonids investigated show degrees of species specificity and reproducibility which enables identification on the basis of a single analysis.

The electrophoretic separation of the salmonid muscle myogens (Fig. 3) was carried out simultaneously to obtain true comparisons of this closely related family of fishes. The close resemblance of *Salmo gairdnerii* with *Salmo clarkii lewisi* in this study and with *Salvelinus fontinalis* and *Salvelinus malma* in a previous study is self-evident. The myogen patterns are virtually independent of sex differences. The apparent difference in the male *O. gorbuscha* is one of concentration. In the genus *Oncorhynchus*, *O. kisutch* and *O. tshawytscha* show identical anodic patterns while the only cathodic difference is that the latter has two additional zones. The relationship of these two species, particularly *O. kisutch*, to the genus *Salmo* is quite apparent. In the other members of the genus *Oncorhynchus*, *O. nerka* and *O. gorbuscha* closely resemble each other, while *O. keta* is clearly intermediate to the two pairs as was also pointed out in a previous communication (Tsuyuki *et al.*, 1962) where the resolutions achieved were not as clear. The myogen patterns of the five species of *Oncorhynchus* strongly suggest that this genus evolved from the *Salmo*, in agreement with previous studies (Tsuyuki and Roberts, 1963) as well as the view of other investigators (Milne, 1948; Neave, 1958; Regan, 1920; Clemens, 1953) utilizing other criteria. The order of evolution of the members of the genus *Onchorhychus* appears to be as indicated in Fig. 3. A study of the mobility characteristics of a specific enzyme enolase, present in the myogen fraction in isoenzymic forms (Tsuyuki and Wold, 1964), clearly shows the manner in which this enzyme has evolved from the genus *Salmo* on through the genus *Oncorhynchus* in the order listed.

In Fig. 4 are shown the multiple and specific nature of the hemoglobin patterns of salmonids. The extent of hemoglobin polymorphism in salmonids, and hence its usefulness phylogenetically, was first noted by the application of starch gel electrophoresis (Tsuyuki and Gadd, 1963). A simultaneous separation on the five species of the genus *Oncorhynchus* was not possible as technical difficulties were encountered in obtaining blood samples at one time. However, it was possible to analyse the males and females at one time and to show that the hemoglobin patterns were independent of sex. The slight difference observed in *O. kisutch* is under investigation. A degree of constancy and species specificity of

patterns similar to that of the myogens were exhibited by the salmonids as well as any other species investigated. However, correlation with the corresponding muscle myogen patterns was not obtained. The close generic resemblance of the latter patterns with existing taxonomic classification was not evident with the hemoglobins. The bony structures and musculatures often used as morphological differences in classification are to a degree reflected at the molecular level in the muscle proteins synthesized. However, phylogenetic information to be found in the hemoglobin molecules, whose major function is that of oxygen transport in fish and other organisms, may be influenced more by the habitat and the availability of oxygen in addition to inter- and intraspecific genetic differences. Water temperatures, aeration, and other factors influencing oxygen availability could conceivably have affected, over the years, the genetic makeup of the synthetic processes involved in the formation of hemoglobin molecules. Previous studies (Vanstone *et al.*, 1964) have already shown that ontogenetic changes take place in the hemoglobins during the life history of the Pacific salmon. Consequently, of the two groups of proteins, muscle myogens yield the more useful phylogenetic information.

A comparison with Fig. 3 shows that the close myogen resemblances between *O. kisutch* and *O. tshawytscha*, as well as between *O. nerka* and *O. gorbuscha*, are not evident in their corresponding hemoglobin patterns except for a few cathodic major zones in the latter pair. However, surprisingly *O. keta* and *O. nerka* differ by only one minor anodic zone, an observation that is at variance with other studies (Clemens, 1953) which indicates that *O. keta* and *O. gorbuscha* are closely related. It is interesting to note that the adults of *O. keta* and *O. nerka* in marine environment are very similar externally prior to the development of their secondary sexual characteristics during river migration.

In Scorpaenidae, nine species were available for study, one from the genus *Sebastolobus* and eight from the genus *Sebastodes*. All the myogen samples were run simultaneously to obtain a direct comparison (Fig. 5A). Generic relationship among the *Sebastodes* is quite evident while *Sebastolobus alascanus* is distinctly different. Of the genus *Sebastodes* examined, *S. flavidus* most closely resembles the genus *Sebastolobus*. Some inconsistency was obtained between the myogen patterns and the identification of *S. ciliatus*[2] and *S. polyspinis*. Consequently, all available specimens of these two species were analysed simultaneously. In Fig. 6, patterns 1, 7, and 10 differ slightly from the others in that the most cathodic zone is less intense and the faint anodic zone third from the origin is extremely faint or absent. These two types of patterns were obtained with both species. The separations achieved on two separate runs, though consistent within themselves, differ in that the second major anodic zone in Fig. 6 has separated into two zones in Fig. 5A. *Sebastodes polyspinis* differs from *S. ciliatus* in having 14 dorsal fin spines instead of 13. Identification based on this characteristic does not quite agree with the myogen patterns as both

[2]Recent information indicates that *S. ciliatus* is a species complex of three or four forms. Unfortunately, taxonomic measurements were not obtained from the specimens studied here. Further morphometric and biochemical studies will be the subject of a later report.

types of patterns were observed in each species. Hemoglobin patterns (Fig. 7A) are likewise nearly identical but the second and third anodic zones are fainter in the one labelled *S. polyspinis*. The protein patterns of these two suggest a single species. Further studies are in progress in an attempt to resolve this apparent anomaly.

The myogen patterns of 11 species from Pleuronectidae run simultaneously are shown in Fig. 5B. Each pattern represents a different genus. The close intergeneric relationships observed in Salmonidae (Fig. 3) and Scorpaenidae (Fig. 5A) are not in evidence. This suggests that the generic classification of Pleuronectidae covers a more diversified range of fishes than in either Salmonidae or Scorpaenidae. In the absence of intergeneric comparisons from other families further discussions on this point are premature. Prolonged frozen storage (6 months) affected the pattern of only one species, *Hippoglossus stenolepis*. In fresh frozen *H. stenolepis*, the most cathodic zone always resolves into three zones of closely similar mobility.

The hemoglobin patterns corresponding to the myogens of Fig. 5A and B are shown in Fig. 7A and B. It is again evident as for the salmonids, that the intergeneric and interspecies relationships found for muscle myogens are not as clearly discernible in the hemoglobin patterns. However, zones of similar mobilities are more in evidence in Scorpaenidae than in Pleuronectidae, supporting the relationships observed for corresponding myogens. With the possible exception of *S. ciliatus* and *S. polyspinis*, species specificity and reproducibility between individuals was maintained for all specimens investigated.

The most obvious exception to the specificity of the myogen pattern was found in *Anoplopoma fimbria* (Fig. 8). Of nine specimens from the Kodiak area of Alaska, seven showed the centre pattern, while the top and bottom patterns were obtained with the remaining two. These fishes co-existed in the same area since they were obtained at the same depth and in the same catch. It is readily seen that the centre pattern includes all the zones of the other two patterns. This is parallel to the results shown by artificially produced F_1 hybrids of the salmonid family (Tsuyuki, unpublished data). Although it is difficult to draw definite conclusions on the basis of a small number of samples examined, extensive natural hybridization may be taking place within *A. fimbria* as was also found in the genus *Oncorhynchus* in an unexpectedly greater frequency than was formerly thought. On the other hand, these observations may indicate the existence of at least three reproductively isolated subpopulations as evidenced further by the presence of yet another variant type of myogen pattern in one sample from the Vancouver area. The hemoglobin patterns of these nine specimens of *A. fimbria* were virtually identical to that shown in Fig. 2. Twenty-four samples of *A. fimbria* obtained subsequently from the Gulf of Alaska confirmed the variations observed in muscle myogen patterns. Although taxonomic measurements of the initial samples are not available, those obtained subsequently are being investigated from this point of view.

The serum proteins of all samples examined were also separated by both starch gel and the polyacrylamide-disc electrophoretic techniques. The latter

technique proved superior to starch gel in resolution. The results of these studies will be described in a later communication.

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NOTES

The Species Specificity and Constancy of Muscle Myogen and Hemoglobin Electropherograms of *Oncorhynchus*

The usefulness of starch gel zone electrophoretic patterns of myogen (Thompson, 1960; Tsuyuki *et al.*, 1962; Tsuyuki and Roberts, 1963; Tsuyuki *et al.*, 1965) and hemoglobins (Manwell *et al.*, 1963; Tsuyuki and Gadd, 1963) in species identification requires that these remain independent of factors other than technical which can influence the mobilities of the protein zones. The genus *Oncorhynchus* has been selected for these studies as specific samples are more readily available. Specimens of each of five species of this genus (*O. kisutch, O. tshawytscha, O. keta, O. nerka, O. gorbuscha*) were therefore analysed to determine the influence of racial, sexual, physiological, and ecological differences on electrophoretic patterns.

At every location, approximately 12 each of male and female, adult fish were sampled. The preparation of samples and the starch-gel zone electrophoretic techniques employed have been described fully elsewhere (Tsuyuki *et al.*, 1962; Tsuyuki, 1963; Tsuyuki *et al.*, 1965). Only one race of *O. keta* was available for study. Samples from both sexes captured at the beginning of their spawning migration at the mouth of the Fraser River (Steveston) and later at their spawning grounds on the Chehalis River showed identical myogen as well as hemoglobin patterns. Both sexes of two runs of *O. gorbuscha*, the "early" and "late" runs, sampled initially at Glen Valley on the lower reaches of the Fraser River and at or near their spawning grounds on the Thompson and Vedder Rivers, respectively, as well as post-spawning samples from the Skeena River system, showed no overall difference in their protein patterns either between or within races. Fully sexually mature *O. kisutch* of either sex from the Green River hatchery in Washington and from selected locations on the Fraser River were identical to pre-puberal ($1\frac{1}{2}$ years) samples (Vanstone *et al.*, 1964) from salt water and to sexually maturing adults from the Skeena River system. However, two groups of fall chinooks (*O. tshawytscha*) sampled at the Green River and Spring Creek hatcheries, and in comparable states of sexual maturity, displayed slight difference in the minor protein zones of their myogen (Fig. 1A) but not in their hemoglobin patterns. The three minor zones just to the left of the origin and clearly shown in all 24 samples obtained from the Spring Creek hatchery were virtually absent or ill-defined in all of the 24 samples obtained at Auburn, Washington. Other consistent differences were found in the relative proximity to each other of the third and fourth zones from extreme cathode end. Of the five species of the genus *Oncorhynchus* analysed, this was the only instance where such differences were observed. However, the slight variations

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in the two groups of chinook salmon are well within the range of specificity of myogen patterns of this species for identification purposes.

No differences in myogen patterns were observed among samples of 650 adult individuals of *O. nerka* from 27 racial stocks of the Pacific Northwest (18 stocks from the Skeena River system, three from the Fraser River system, two from Alaska, and one each from the Nass River, Smith's Inlet, River's Inlet, and Baker Lake, Washington), providing convincing evidence that muscle myogen and hemoglobin patterns are essentially invariable species-specific properties of this species. The overall electrophoretic properties of these proteins, therefore, are not affected by differences in sex, sexual maturity during the period of spawning migration, or by the slight variations in environmental conditions at their spawning grounds. Samples captured at sea also showed identical patterns. Furthermore, a single analysis from one fish suffices to establish identification while much larger samplings are required for identification by meristic characters (Rounsefell, 1962).

Although slight variations in the minor protein zones were observed, the myogen patterns from yearling specimens of *O. nerka* fell within the range characteristic for the corresponding adults (Fig. 1B). The hemoglobin patterns, however, were subject to ontogenetic changes as described in another communication (Vanstone *et al.*, 1964). Two of the three discrete yearling *O. nerka* samples from the Babine Lake system appeared to show slightly different myogen patterns.

Instances of myogen pattern variations observed in other species of fishes as well as the occasional samples of *Oncorhynchus* have been discussed in relation to natural and artificial hybridization studies (Tsuyuki *et al.*, 1965). The results of this study emphasize the usefulness of myogen and hemoglobin patterns for phylogenetic purposes.

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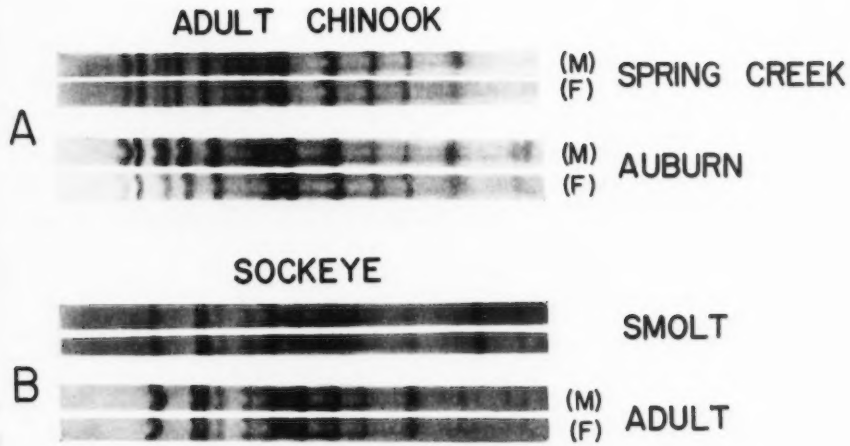

FIG. 1. A. Starch-gel electropherograms of the muscle myogens of fall chinooks obtained at the Spring Creek hatchery on the Columbia River, and at the Green River hatchery at Auburn. (M) and (F) stand for male and female, respectively. All the samples were obtained from fish immediately following artificial spawning. The anode is to the right and the cathode to the left. B. Starch-gel electropherograms of the muscle myogens of smolt and adult sockeye salmon. The smolts were obtained from the Babine Lake system and the adults from the Lower Adams River during egg taking.

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Fin Anomalies in the White Sucker, *Catostomus commersoni*, Population in Heming Lake, Manitoba

Structural aberrations in fishes are not seen as frequently in nature as in hatcheries, possibly because a large percentage are so abnormal that they do not survive. In the population of white sucker, *Catostomus commersoni*, in Heming Lake, Manitoba, complete absence of individual pectoral, pelvic, and dorsal fins have been noted at various times. The most recent and certainly the most striking example of abnormal fin development is illustrated in Fig. 1. The high "grayling-like" dorsal fin was readily visible to the investigator and

TABLE I. Comparison of fin measurements of an unusual sucker and normal suckers of the same length from Heming Lake, Manitoba.

	Abnormal	Normal
	mm	*mm*
Height of dorsal fin	96.3	38.4
Height of anal fin	74.0	45.1
Length of ventral fin	59.0	39.0
Length of pectoral fin	86.0	46.0

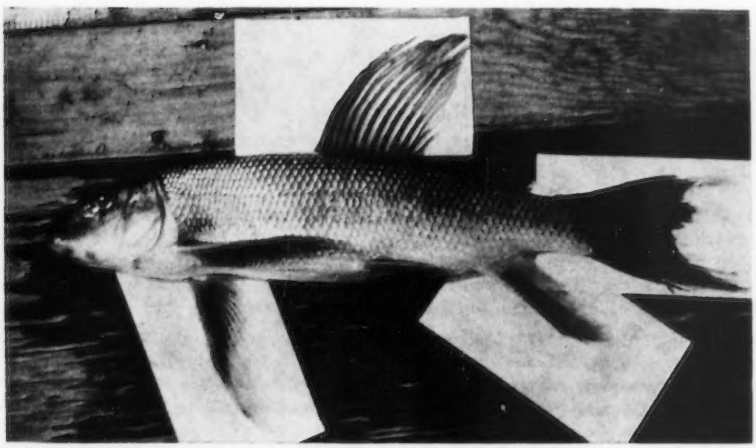

FIG. 1. Photograph of Heming Lake white sucker, *Catostomus commersoni*, with unusually long fins (fork length 284 mm).

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further examination showed that all fins without exception were much longer than normal. This abnormal fish was 4 years old and was 284 mm in fork length (standard length 325 mm). In Table I its measurements are compared with the average measurements of 30 or more normal suckers between 250 and 300 mm in length. The dorsal fin was 2.5 times as large as the normal, the pectoral fin 1.9 times, the anal fin 1.6 times, and the ventral fin 1.5 times. Its ray counts differed little from the average for normal suckers in this size range and are not shown.

The long trailing extremities of this fish gave it an exotic tropical-fish appearance and caused it to stand out among others of its species. The fact that the fins, with the exception of the caudal, were not frayed was interesting to note, as one would expect that these elongated appendages would attract predators. As this fish was in its 5th year of life at the time of capture, it would appear that this mutation had not affected its chance of survival.

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Retinal Structure in the Arctic Char (*Salvelinus alpinus* L.)

In the summer of 1963, Mr J. G. Hunter of the Fisheries Research Board of Canada Arctic Biological Station kindly placed at my disposal 20 specimens of arctic char which came in two batches. The first batch (6–11 cm) was from the mouth of the Sylvia Grinnell River in the Frobisher Bay region. The second batch which consisted of larger fish, including an adult female 12–15 years old (25 cm), came from a landlocked lake (Keyhole Lake) on Victoria Island (69°23′N–116°13′W). The temperature of these bodies of water from which these fish were taken was 8–10°C. They were transported by air in thermos containers and on arrival in Montreal the temperature of the water in the containers was 10°C. The fish were transferred to aerated, refrigerated water at 10°C. They were thus held in the laboratory for 10 days. During this period they refused to feed and five smaller fish died. Since these fish are native to arctic waters and hence adapted to an environment which is quite different from that of the other salmonids hitherto studied, it was felt that it would be of interest to study the structure of their retina and its photomechanical responses.

Five fish were sampled in light (50 ft-c) during the day and five in dark during the night. They were transferred to Bouin's and soon after a puncture was made in the cornea to facilitate the fixation of the retina. The eyes were enucleated after 48 hr, the corneas and the lenses removed, and the rest dehydrated in Dioxan and imbedded in paraffin (61°C m.p.). Sections 3 or 5μ thick were cut, stained with Harris' haematoxylin, and counterstained with alcoholic eosin. Some sections were examined unstained, with a phase contrast microscope.

It is interesting to see that in its structure the retina of the arctic char is remarkably similar to those of the *Oncorhynchus* spp. (Brett and Ali, 1958; Ali, 1959), *Salmo salar* (Ali *et al.*, 1961) and *Salvelinus fontinalis* (Rochon-Duvigneaud, 1943). It consists of the usual eight layers and two membranes (Fig. 1).

1. *Retinal pigment epithelium.* It is very well developed and contains granular pigment which disperses fully in light (Fig. 1) and contracts in dark (Fig. 2). In the light-adapted state the pigment extends to the tips of the fingerlike processes of the epithelial cells. In the dark-adapted state it is concentrated proximally forming a dense strip with numerous slender protrusions. In this state the width of the strip is about half of that in the fully dispersed condition.

2. *Visual cell layer.* This layer is composed of rods and cones. The former are slender and are expanded in the light-adapted condition and lie in between the epithelial cell projections into which the pigment has expanded (Fig. 1). In the dark-adapted state they are contracted and lie close to the external limiting membrane (Fig. 2). The cones are prominent and are mostly single with a few equal twins. In the light-adapted condition they are contracted (Fig. 1) while in the dark-adapted state they are expanded and are situated close to the contracted epithelial pigment (Fig. 2). The single as well as the equal twin cones show the same degree of photomechanical response. As is usual, the central and ventral regions of the retina contain more cones

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per unit area than the dorsal region. The cones in the dorsal region appear to be bulkier. The dorsal region contains a larger number of rods.

3. *External limiting membrane.* This separates the visual cell layer from the external nuclear layer and is very clearly seen.

4. *External nuclear layer.* This layer consists of four layers of rather well-packed nuclei of the rods and cones. In the accompanying photographs (Fig. 1 and 2) it is possible to locate the connexions between some of the visual cells and their nuclei. The nuclei are small and darkly stained and it is not possible to distinguish between the rod and cone nuclei.

5. *External plexiform layer.* This is a layer loose in texture. In this layer the synapses of the foot-pieces of the rods and cones with the neurons of the inner nuclear layer occur. The foot-pieces of cones are seen as darkly stained, large plates while those of the rods are lightly stained and smaller and form a row just below the external nuclear layer (Fig. 1).

6. *Internal nuclear layer.* As is the case in a cone-rich retina this layer is wider than the external nuclear layer. Two groups of nuclei forming this layer can easily be recognised. The smaller, darkly stained nuclei are situated externally, i.e. towards the external plexiform layer. They are densely packed four or five deep. These are bipolar cells (centrifugal, diffuse, horizontal, and midget bipolar cells) and a few nuclei of the Müller's supporting cells. The other nuclei forming this layer are larger and less darkly stained. They lie more inwardly, i.e. towards the internal plexiform layer, and form a zone about four nuclei deep. These are the amacrine cells. These two groups of cells are not separated, i.e. the internal nuclear layer is a unified layer. In the middle part of this layer one or two rows contain both types of nuclei.

7. *Internal plexiform layer.* This is a very wide, well-defined layer, uniformly dense, and virtually free of cells. It is broader than the two preceding layers and the synapses between the cells of the internal nuclear layer and the ganglion cells occur in this layer.

8. *Ganglion cell layer.* The large, fairly numerous and lightly stained ganglion cells form a single row. This is typical of a cone-rich retina.

9. *Nerve fibre layer.* This layer is made up of the nerve fibres from the ganglion cells. As is usual it is thicker in the central part of the eye than in the peripheral regions.

10. *Internal limiting membrane.* This membrane which forms the innermost boundary of the retina is clearly seen in this retina.

Thus it is seen that this retina is remarkably similar to that of the other salmonids examined before. The relationship between the thickness of the retina, thickness of the pigment epithelial layer (in the light-adapted condition), length of the cones, number of cones and ganglion cells in a unit area of the retinal section, and the length of the fish and the diameter of its eye is similar to that observed in the Atlantic salmon, *Salmo salar* (Ali, 1963, 1964). The well-developed pigment epithelium, large number of prominent cones, numerous rods, well-developed internal nuclear and plexiform layers, prominent and numerous ganglion cells, and the ability of the pigment epithelium, cones, and rods to undergo striking photomechanical changes indicate a retina which is well suited for photopic as well as scotopic vision.

This work was carried out in the Université de Montréal with financial assistance from the National Research Council of Canada. Mr Rod Stevenson assisted in technique and Professor P. K. Menon kindly read the manuscript.

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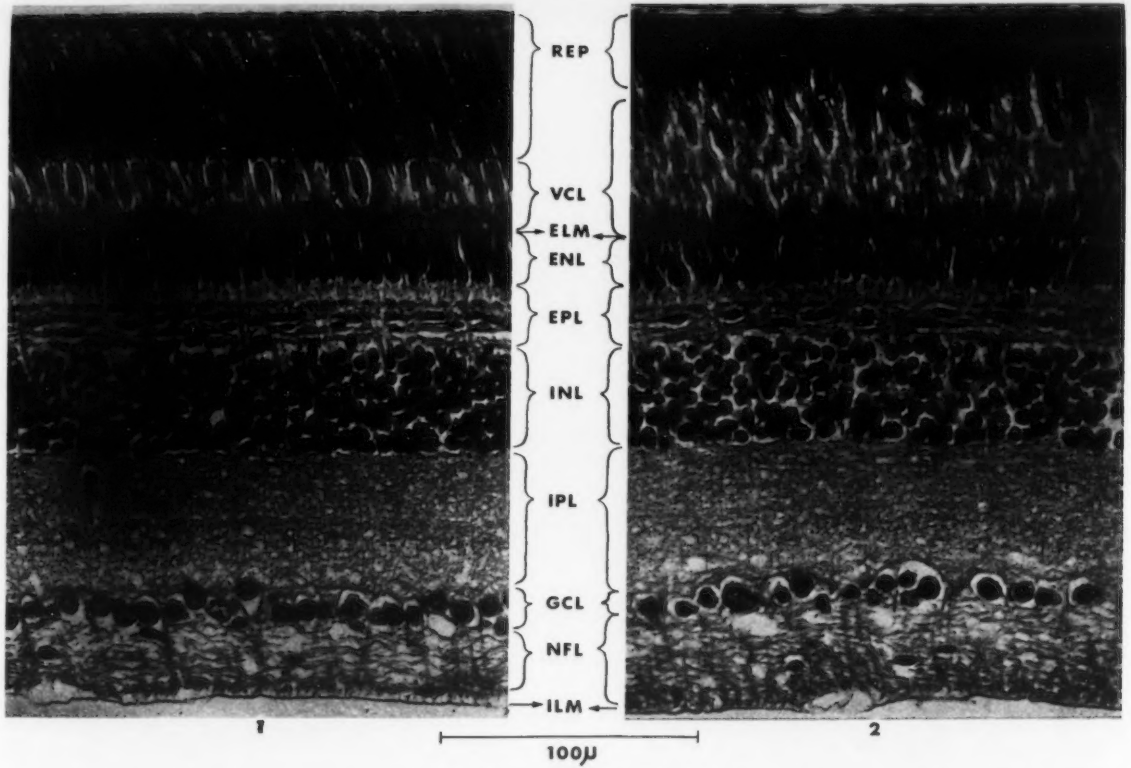

FIG. 1. Photomicrograph of a cross section (5 μ) of a light-adapted retina. REP — Retinal pigment epithelium; VCL — Visual cell layer; SC — Single cone; TC — Equal twin cone; R — Rod; ELM — External limiting membrane; ENL — External nuclear layer; EPL — External plexiform layer; FP — Foot-piece of rod or cone; INL — Internal nuclear layer; IPL — Internal plexiform layer; GCL — Ganglion cell layer; NFL — Nerve fibre layer; ILM — Internal limiting membrane.)

FIG. 2. Photomicrograph of a cross section (5 μ) of a dark-adapted retina. Note the contracted pigment and rods, and the expanded cones (abbreviations as in Fig. 1).

Ali — J. Fish. Res. Bd. Canada

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Current Observations in Fury and Hecla Strait

On September 11 and 12, 1960, observations of current speed and direction were made in CSS *Baffin* while moored at station 45 in Fury and Hecla Strait. Observations were generally made at hourly intervals at 10, 20, 30, and 50 m depth using an Ekman (1932) current meter, and at the surface using a captive vane or drogue. The data were observed during a period the vessel was engaged in a hydrographic survey of a section of Fury and Hecla Strait, so that tidal height data are available (Fig. 1A). The current observations extended over a 36-hr period, but due to an inadequate mooring the initial and major portion of the data strongly reflect movement of the ship. For a period of about 13 hr the mooring was improved so that realistic data concerning speed were obtained. In observations of direction it is at 50 m only that the current meter data appear free of deviation due to the presence of the steel ship. Speed and direction at this depth and the observations at shallower depth are presented in Fig. 1B, and are available in tabular form in a record of the Canadian Oceanographic Data Centre (1964), which also includes temperature and salinity data observed in Fury and Hecla Strait shortly before and after station 45 was occupied.

At each depth a speed maximum (about 30 cm/sec) occurred about 0200 GMT on September 12, with another suggested toward 1400. A secondary maximum is indicated as having occurred at 0800 about mid-way of a period in which the current strength was relatively weak, or in which a slack water occurred. At 50 m the maximum speed appears to be associated with a direction close to east, and the secondary maximum with a direction about west. Dohler (1964) interpreted the tidal height data and described the tide here as "mixed, mainly diurnal." It appears then that the eastward movement is associated with the rise to a higher high water, while the lesser westward movement is associated with a fall to a higher low water.

With the speed and direction data at 50 m, 13 hourly values were obtained (Fig. 2) and utilized to provide an assessment of the net or residual current. Over an assumed semidiurnal cycle of about 12.5 hr this was determined to be 8 cm/sec in direction 085° true. If this residual is assumed to have occurred throughout the section including station 45, then a net volume displacement of 0.1×10^6 m^3/sec to the eastward out of Fury and Hecla Strait was taking place.

Campbell (1958) remarked that the influx of water from Fury and Hecla Strait into Foxe Basin has been calculated to be 0.05 to 0.1×10^6 m^3/sec. In a summary of conditions of current, Collin (1958) indicated that current reversals of tidal period were to be expected, and that these could be obscured by an increase in strength of a residual current from the westward. Advice in "Pilots" of the Canadian Hydrographic Service (1959) reaffirmed the existence of the residual and notes, as well, that a possible current maximum of 2 knots to the eastward can occur. This apparently would be experienced in the section

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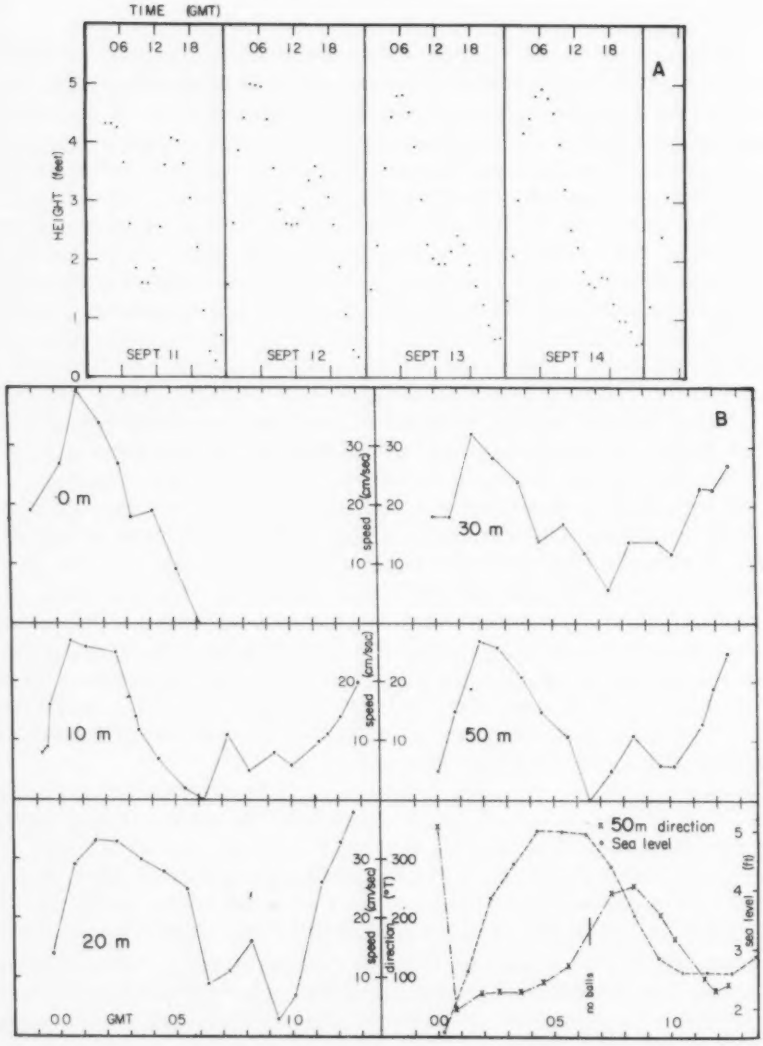

FIG. 1, A. Variation of sea level at Purfur Cove, Fury and Hecla Strait September 11–14, 1960, based on a reduction to hourly values provided by the Canadian Hydrographic Service. B. Current speed at 0, 10, 20, 30, and 50 m with direction at 50 m at Station 45, and sea level at Purfur Cove September 12, GMT.

Time (*GMT*)	Speed (*cm/sec*)	Direction (°*T*)	Hour (*No.*)
00	05	350	1
01	18	050	2
02	27	070	3
03	24	070	4
04	19	080	5
05	13	100	6
06	06	150	7
07	03	220	8
08	09	250	9
09	08	220	10
10	06	180	11
11	11	120	12
12	21	080	13

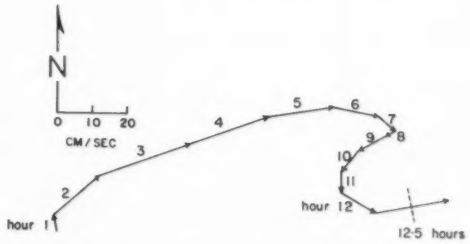

FIG. 2. Tabulation of hourly values of speed and direction at 50 m and presentation in a vector diagram from which residual estimated.

including Labrador Narrows, the narrowest point in Fury and Hecla Strait where the area (0.0741×10^6 m^2) is over an order of magnitude less than that (1.17×10^6 m^2) in the section including station 45. On the basis of the calculated net movement a residual in Labrador Narrows could be expected of at least 80 cm/sec. This combined with a possible tidal current of the intensity observed at station 45 could lead to maximum values to the eastward somewhat greater than the 2 knots (100 cm/sec) mentioned. Thus the present data lend credence to the earlier observations.

Of additional interest is an apparent secondary effect of tidal currents on the distribution of the summer heat input. Observations of temperature in September 1960 indicate that relatively warm water occurred to at least 100 m within Fury and Hecla Strait, while those of September 6 (Fig. 3) indicate not only this warm water but also the relatively cold water throughout the depth to the westward in the Gulf of Boothia. Comparison of the heat input which might have occurred in Fury and Hecla Strait, assuming conditions of ice cover and insolation, with the extent of heat storage, represented by the difference between the observed temperature and that likely occurring in winter, suggests that a warm water was being accumulated there through advection. In this region warm water is surface water, so the implication is that the water accumulated was predominately a surface water. Campbell and Collin (1958) and Campbell (1964) remarked on some of the oceanographic consequences of the extensive area of shallow water and tide flats in Foxe Basin particularly in the eastern and northern sections. Here a relatively large proportion of the total amount of water is necessarily surface water. Under the assumption that equal amounts of water from the eastward and from the westward participate in the purely tidal activity in Fury and Hecla Strait it follows that a significant portion would derive from this surface water, and could, in summer, lead to the existence of the observed warm water.

That such an effect could occur even though significant residual current likely existed suggests the possibility that heat gained in summer in Foxe

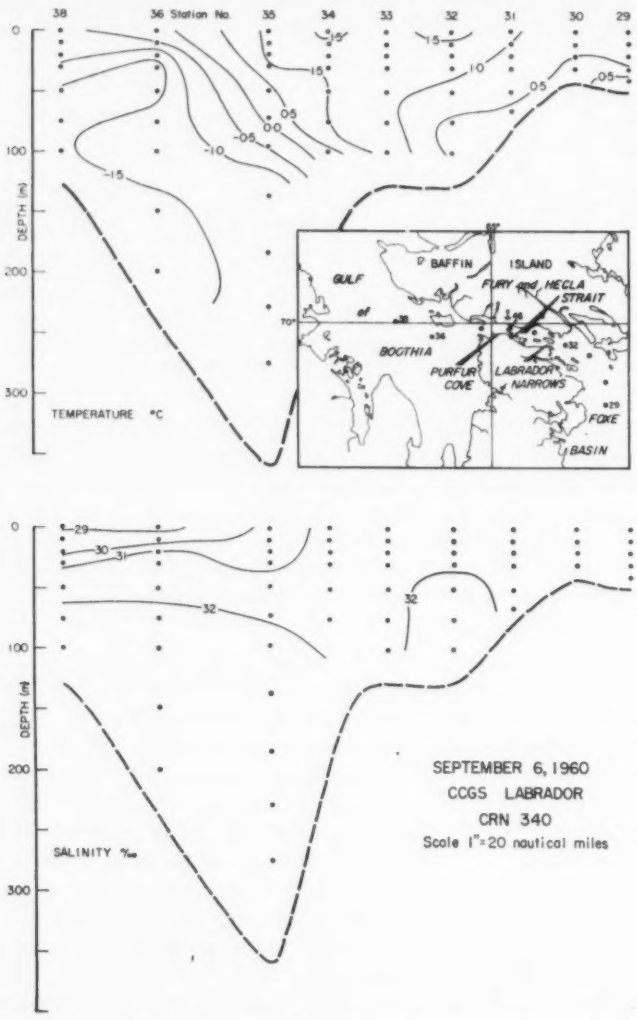

FIG. 3. Serial temperature and salinity data in a section through Fury and Hecla Strait and location of stations, September 6, 1960. These data have been reported (CODC, 1964).

Basin may influence the extent of ice cover in and westward of Fury and Hecla Strait.

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Northern Range Extensions for Four Species of Rockfish (*Sebastodes goodei*, *S. helvomaculatus*, *S. rubrivinctus*, and *S. zacentrus*) in the North Pacific Ocean

Early in 1963, the Fisheries Research Board's new research vessel CGS *G. B. Reed*, began operations in the North Pacific Ocean. Through August 1964 the vessel had completed five groundfish trawling expeditions extending from Vancouver Island, British Columbia, to Unalaska Island, Alaska. Four species of rockfish (Family Scorpaenidae) not previously recorded from the area of capture were noted. One of these is a new record for British Columbia waters.

Sebastodes goodei Eigenmann and Eigenmann 1890 Chilipepper

On February 25, 1964, the author collected a rockfish identified as *Sebastodes goodei* off the northwest coast of Vancouver Island, British Columbia, 40 miles southwest of Cape Scott (50°39.5′N lat; 128°39.5′W long). The fish, a female with fork length 352 mm (13.9 inches) (Fig. 1), was caught with a 400-mesh, semi-balloon, groundfish trawl in 95–96 fathoms. This is the first record of the chilipepper rockfish in British Columbia waters.

S. goodei resembles the Pacific ocean perch, *S. alutus*, in colour and body shape, but is readily distinguishable from the latter species by the presence of white peritoneum, 60–77 diagonal rows of scales below the lateral line, and the absence, in adults, of all spines on the top of the head. The British Columbia specimen differs from those in California in that the maxillary extends well past the mid-point of the orbit. Hence, Phillips' (1957) key proved to be inapplicable.

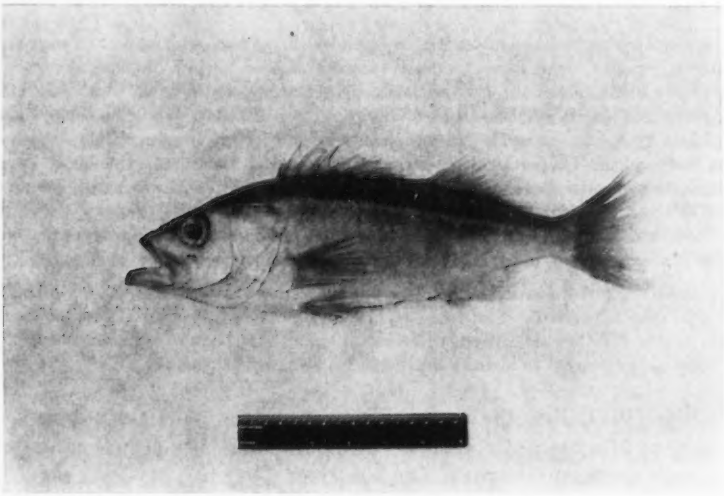

FIG. 1. Photograph of *Sebastodes goodei*.

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Phillips (1957) lists the range as Magdalena Bay, Baja California, to Eureka, California. Alverson *et al.* (1964) reported catches in the Oregon–Washington region (California–Oregon border to Cape Flattery, Washington) but did not give specific locations. The known range of this species is thus documented to extend northward approximately 600 miles from Eureka, California.

The specimen has been deposited in the museum of the Institute of Fisheries, University of British Columbia (catalogue No. BC 64-283).

Sebastodes helvomaculatus (Ayres 1859) Rosethorn rockfish

On July 25, 1963, the author collected three rockfish specimens, identified as *Sebastodes helvomaculatus*, in the Gulf of Alaska, 65 miles east of Cape St. Elias (59°33′N lat; 142°34′W long). The fish, all males, had fork lengths of 275 mm (10.8 inches), 312 mm (12.3 inches), and 314 mm (12.4 inches), respectively. They were caught with a 400-mesh, semi-balloon, groundfish trawl in 73 fathoms.

A number of specimens of the rosethorn rockfish were also caught, during 1963 and 1964, by the *G. B. Reed* in British Columbia waters — 20 from Queen Charlotte Sound and 10 from the west coast of Vancouver Island (Estevan Deep). Since Clemens and Wilby (1961) did not list this species, these were thought to be new records for British Columbia waters. However, N. J. Wilimovsky (personal communication) has reported that the single specimen of *S. rosaceus* reported by Clemens and Wilby subsequently has been identified as *S. helvomaculatus*, albeit an "aberrant" form.

The rosethorn rockfish resembles the rosy rockfish, *S. rosaceus*, with respect to morphometric ratios, meristic counts, head spines, and the presence of 4 or 5 white blotches along the back. Phillips (1957) distinguishes the two primarily by the regression of orbit width into standard length on standard length, and body colour. Heyamoto and Hitz (1962) failed to achieve a separation for their 14 specimens using the morphometric ratio regression on standard length, and relied on body colour. The author likewise could not confirm identification of his 33 specimens (and the single, redescribed specimen of *S. rosaceus*) with the morphometric ratio. Figure 2 shows Phillips' (1957, fig. 2) original regression lines for *S. helvomaculatus* and *S. rosaceus*, together with the morphometric values obtained for the aforementioned 34 specimens. It is evident that no clear separation can be obtained, particularly for the smaller specimens.

A complete record of morphometric ratios and meristic counts was compiled for 24 specimens, and did not differ appreciably from those reported by Phillips (1957) for California specimens. However, neither did they differ from those for *S. rosaceus*. Reluctantly, the author could only rely on body colour pattern to identify his specimens as *S. helvomaculatus*.

Phillips (1957) lists the range of this species as Guadalupe Island, Baja California, to Monterey, California. Heyamoto and Hitz (1962) extended the known range northward to the Columbia River (Oregon). Alverson *et al.* (1964) reported catches in the Gulf of Alaska region (Cape Spencer to western Kodiak Island) without giving specific locations. The present record extends the known range northward approximately 700 miles from the Columbia River.

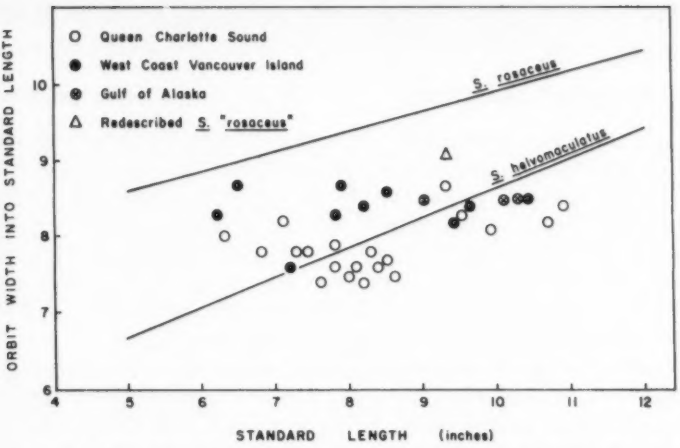

FIG. 2. Regression of orbit width into standard length on standard length for 34 specimens of *Sebastodes helvomaculatus* collected in British Columbia and Alaskan waters. (Regression lines are from Phillips (1957), figure 2, p. 14.)

The specimens have been deposited in the museum of the Institute of Fisheries, University of British Columbia (catalogue Nos. BC 64-294, 295, 296, 297, and 298).

Sebastodes rubrivinctus (Jordan and Gilbert 1880) Flag rockfish

On August 2, 1964, the author collected two rockfish specimens, identified as *Sebastodes rubrivinctus*, 65 miles southwest of Kodiak Island, Alaska (55°42′N lat; 155°09′W long). The specimens were caught in a 400-mesh, semi-balloon, groundfish trawl fished in 104–110 fathoms. The fork lengths were 283 and 286 mm, respectively. Sex was not determinable from the thawed fish when examined at this Station.

S. rubrivinctus is readily identified by the four red (or pink) vertical bars on the pink (or white) body, the absence of the supraocular, coronal, and nuchal head spines, and the number of gill rakers on the first gill arch (26–32).

Morphometric ratios and meristic counts were compiled as in Phillips (1957). Minor differences (± 0.1) in the ranges of morphometric ratios were noted for nine of Phillips' categories. Orbit width into head length was the only ratio to display an appreciable difference. The Alaska specimens had a range of 3.4–3.5 whereas Phillips (1957, p. 93) reports 3.6–4.3 for California fish. None of the meristic count ranges (or physical descriptions) deviated from those reported by Phillips.

Phillips (1957) listed the geographical range for the flag rockfish as Santo Tomas Bay, Baja California, to Southeast Alaska. Heyamoto and Hitz (1962) reported catches of this species off the Copper River (Gulf of Alaska), 300 miles to the westward of Southeast Alaska (Cape Spencer). The present record documents an extension of the known northern range of this species westward approximately 400 miles from Copper River.

The two specimens have been deposited in the museum of the Institute of Fisheries, University of British Columbia (catalogue No. BC 64-452).

Sebastodes zacentrus (Gilbert 1891) Sharpchin rockfish

On July 25, 1963, the author collected specimens of *Sebastodes zacentrus* from a groundfish trawl catch in the Gulf of Alaska, 65 miles east of Cape St. Elias (59°39′N lat; 142°27′W long), in 98–103 fathoms. Subsequently, additional specimens were caught westward to a point off the east coast of Sanak Island (54°13′N lat; 161°37′W long) on August 12, 1964, in 52 fathoms. Trawl catches of the sharpchin rockfish throughout this area ranged from 1 fish to 2600 lb per 30-min haul. The depth range of catches was 52–130 fathoms, and the size range of specimens, 60–321 mm (2.4–12.6 inches), fork length.

On November 4, 1963, two specimens of *S. zacentrus* were collected by W. E. Barraclough in northeast Hecate Strait, British Columbia, from a midwater trawl catch of the *G. B. Reed* in Freeman Passage (53°50.1′N lat; 130°48.2′W long) at 50–62 fathoms (the trawl was fished near the bottom). Fork lengths were 110 mm (4.3 inches) and 111 mm (4.4 inches), respectively; sex could not be determined.

During February and March 1964, additional specimens were collected with a groundfish trawl in Queen Charlotte Sound, Smith Sound (southeast Queen Charlotte Sound), and off the northwest coast of Vancouver Island.

The sharpchin rockfish closely resembles the stripetail rockfish, *S. saxicola*, lacking a supraocular spine and possessing a second anal spine longer and thicker than the third. It is distinguished from *S. saxicola* by lacking two strong spines on the lower margin of the suborbital bone, and possessing a "shelf" beneath each nostril which is formed by the suborbital bone.

Morphometric ratios and meristic counts for 68 Alaska specimens from Cape St. Elias to Sanak Island were obtained as in Phillips (1957). Ratios and counts which differed appreciably from those reported by Phillips for California specimens are shown in Table I, together with the California data. Head length, body depth at ventral fin origin, upper jaw length, and longest dorsal fin ray were generally shorter for Alaskan specimens. Ventral and dorsal lengths at the caudal peduncle appeared to be longer for Alaska fish, but results are inconclusive. Meristic counts were generally higher for the Alaskan specimens and increased westward. For example, diagonal scale row counts were 45–55 (30 specimens) near Cape St. Elias (142° W long), 50–54 (13 specimens) at Chirikof Island (155°W long), and 54–59 (19 specimens) at Sanak Island (162°W long). Phillips reported 43–50 for 15 California specimens.

Phillips (1957) reports the range of *S. zacentrus* as San Diego, California, to southern British Columbia. Alverson *et al.* (1964) report catches in the southeast Alaska region (Dixon Entrance to Cape Spencer). For British Columbia waters, Clemens and Wilby (1961) report specimens collected in Burrard Inlet (Vancouver), Hardy Bay (Queen Charlotte Strait), and Hope Island (northern tip of Vancouver Island). Hitz *et al.* (1961) reported catches of *S. zacentrus* in the vicinity of Cape Scott (Vancouver Island). This species appears to be well distributed throughout British Columbia waters. Specimens captured in the Gulf of Alaska represent an extension of the known range northward approximately 1000 miles north and westward from British Columbia.

TABLE I. Comparison of morphometric ratios and meristic counts for specimens of *Sebastodes zacentrus* collected in California and Alaska.

Measurement	Area	
	California[a]	Alaska[b]
	Measurement into standard length	
Head length	2.7–2.8	2.6–3.3
Body depth at ventral fin origin	2.7–3.2	2.7–3.7
Upper jaw length	5.9–6.4	5.9–7.6
Longest dorsal fin ray	6.6–7.1	6.2–9.2
	Measurement into head length	
Ventral length of caudal peduncle	1.8–2.0	1.3–2.1
Dorsal length of caudal peduncle	2.8–3.1	1.8–3.1
	Orbit width into measurement	
Ventral length of caudal peduncle	1.5–1.8	1.5–2.4
Dorsal length of caudal peduncle	0.9–1.2	1.0–1.7
Rakers on first gill arch	33–37	34–40
Pores on lateral line	39–45	41–51
Diagonal scale rows below lateral line	43–50	45–59

[a]Phillips (1957, p. 103) — 15 specimens, 165–335 mm.
[b]Yakutat to Sanak Island — 68 specimens, 60–321 mm.

The 19 Sanak Island specimens have been deposited in the museum of the Institute of Fisheries, University of British Columbia (catalogue No. BC 64-453).

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Some Abnormalities in Skates (*Raja*) of the Newfoundland Area

In the course of fisheries research in the Newfoundland area since 1946, a number of abnormalities have been noted in skates, especially in the thorny skate, *Raja radiata*, which is the most common skate in the area. Some of the more unusual of these abnormalities are presented here.

Separation of pectoral from the head. In November 1958 we received an immature male thorny skate, *Raja radiata* Donovan, with the pectoral fins separated from the head, but otherwise normal (Fig. 1). This is the only abnormality of this type that we have noted in 18 years of observations of Newfoundland skates.

This abnormality develops from the failure of the anterior lobes of the pectorals to join with the head during embryonic development. Although the abnormality must be rare in the Newfoundland area it is so noticeable that such skates are likely to be observed by fishermen and brought to the attention of biologists. Thus there are in the literature many (usually single) records of skates and rays with anteriorly notched pectorals. Among others it has been noted by Saemundsson (1909) for *Raja radiata*, Johnstone (1906) for *Raja clavata*, Radcliffe (1928) and Gudger (1933) for *Raja laevis* (as *stabuliforis*), and Letaconnoux (1949) for *Raja brachyura*. Gudger (1933) also gives a review of a long history of such malformations dating back to 1810 and probably much earlier. Many skates and rays malformed in this way were given new generic and specific names. For literature and discussion of this type of abnormality see also Bigelow and Schroeder (1953) and Letaconnoux (1949).

Tail abnormalities. Absence of the tip of the tail is not unusual in skates, and in some cases could be the result of abnormalities in development. Usually it gives the appearance of being cut off or more probably bitten off by a shark or dogfish, or by some other fish when the skate was young (see example of *Raja jenseni* in Templeman, MS, 1964). Two individuals of *Raja radiata* have been noted with only the base of the tail remaining. One of these was taken on the Northeast Newfoundland Shelf (Fig. 2). The other, an immature male with a calculated total length to tip of tail of 46 cm, was taken by the longliner, *Atlantic Rover*, in September 1953 off Battle Harbour, Labrador, in 265 m, at 52°12′15″N and 54°18′30″W. In both of these skates the skin had grown completely over the stub of the tail.

Abnormalities of the vertebral column. Occasionally, as in other fishes, a skate is observed with a noticeably curved vertebral column (Fig. 3). We have noted at least two *Raja radiata* with a distinct abnormality of this type, one illustrated in Fig. 3 and the other a mature male 84 cm long taken on St. Pierre Bank on May 16, 1962. These vertebral column abnormalities are presumably produced during embryological development.

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Snout abnormality. A barndoor skate, *Raja laevis* Mitchill, was observed in which the rostral cartilage did not extend far enough forward to produce the usual pointed snout of this species. The junction of the pectorals with the shortened snout during embryonic development apparently produced the blunt-snouted condition shown in Fig. 4.

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NOTE: Fig. 1–4 follow.

FIG. 1. Left, dorsal and right, ventral views of a thorny skate, *Raja radiata*, male, immature, total length 63 cm, caught by otter trawler *Gaultois*, November 1958 on the southern Grand Bank at 43°26′N, 50°18′W, 55 m. (Photographs of Fig. 1–4 are by E. L. Rowe.)

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FIG. 2. Dorsal view of a thorny skate, *Raja radiata*, female, immature, total length (calculated to tip of normal tail) 48 cm, caught by the *Investigator II* September 1, 1952, at 51°12'N, 54°30'W, 220–268 m, bottom temperature 0.38 to 0.55°C.

FIG. 3. Dorsal view of a thorny skate, *Raja radiata*, male, mature, caught by the *Investigator II*, December 7, 1953 in Conception Bay, Newfoundland at 265 m, bottom temperature –0.89°C.

FIG. 4. Dorsal view of a barndoor skate, *Raja laevis*, male, mature, total length 138 cm, caught by the *Investigator II*, June 12, 1947 on the southwestern Grand Bank at 44°16′N, 52°18′W, 93 m, bottom temperature 5.16°C.

The Salinity of Baker Lake, N.W.T., Canada

Baker Lake is situated at the head of Chesterfield Inlet, which stretches inland approximately 100 miles from the west coast of Hudson Bay, 350 miles north of Churchill, Manitoba. The lake is formed by a sill at the head of the inlet which is covered with a minimum depth of 5.5 m of water at mean low water springs. Small cargo ships from Churchill regularly make the passage up to the settlement at the head of the lake.

Sampling was carried out through 2.4 m of ice in the position 64°7.4′N lat, 94°46.4′W long, in the area of deep water indicated on the navigational chart for the area (Canadian Hydrographic Service chart no. 5439) on April 24, 1964. The samples were obtained with a weighted polythene bottle from which the cork could be removed at the appropriate depth by jerking the line.

The primary object was to ascertain the salinity of Baker Lake waters. Johnson (1964) had suggested that the lake might have a saline layer on the bottom because of the presence of *Mysis litoralis* Holmquist and the marine form of *Gammaracanthus loricatus* Sabine in stomach contents of lake trout (*Salvelinus namaycush*) from the mouth of the Prince River.

The results of the analysis of the water samples (Table I) show a marked increase in the content of all constituents below a depth of 20 m, and the high proportion of magnesium compared with that of calcium indicates the presence of sea water. The increase of chloride ion content with depth is plotted in Fig. 1.

It is apparent that Baker Lake has a bottom layer of very dilute sea water since normal sea water has a chloride ion content in the region of 17,700 ppm whereas the maximum in Baker Lake was found to be 342 ppm at a depth of 60 m. The content of all constituents increases rapidly below 20 m although even the surface waters show a high proportion of magnesium to calcium, indicative of considerable mixing with the lower layers.

Three possible explanations for the presence of this sea water are: firstly, with the rising of the land surrounding Hudson Bay (Lee, 1960) a quantity of sea water has been trapped in the bottom layer of water since the time when the lake was a genuine arm of the sea; secondly, sea water seeps through the rocks forming the sill at the outlet of the lake; and finally, there is an occasional influx of the sea over the sill. In view of the relatively shallow depth of the lake, the extreme dilution of the sea water, and the mixing found in the surface layers, it seems unlikely that the water can be of ancient origin; such a system would be very unstable and the salt would disappear rapidly from it. Underground seepage is also unlikely in view of the permafrost closing any access that might otherwise occur. The most likely solution is that an incursion of the sea takes place when exceptionally high tides occur, probably combined with an easterly wind. The highest tides in this region rise 2.44 m above mean sea level whereas the surface of the lake, according to the topographical map of the area, is 1.52 m above sea level; the physical circumstances thus by no means preclude an invasion of the lake by the sea.

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TABLE I. The analysis of water samples from various depths in Baker Lake.

	Depth (*m*)						
	0	10	20	30	40	50	60
pH	6.7	7.0	6.9	7.0	6.9	7.0	6.8
Alkalinity (total)	8.2	7.6	6.9	7.0	8.0	9.0	7.2
Specific conductance	38.6	47.8	60.7	334	690	969	1185.0
Hardness as (total)	11.6	10.9	11.9	36.8	66.7	95.0	119.0
$CaCO_3$ (non-carbonate)	3.4	3.3	5.0	29.8	58.7	36.0	112.0
Calcium	1.7	1.4	1.4	1.8	2.1	4.1	5.0
Magnesium	1.8	1.8	2.0	7.8	14.9	20.6	25.9
Sodium	2.7	3.8	5.7	47.0	96.0	141.0	185.0
Potassium	0.8	0.5	0.5	2.2	4.1	6.2	7.3
Carbonate	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Bicarbonate	10.0	9.3	8.4	8.5	9.8	11.0	8.8
Sulphate	1.8	1.6	4.1	13.6	26.2	39.0	47.5
Chloride	5.6	7.6	11.3	89	183	268	342
Fluoride	0.1	0.09	0.09	0.09	0.13	0.13	0.13
Phosphate	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nitrate	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Silica	0.2	0.0	0.2	0.1	0.1	0.1	0.2
Sum of constituents	19.6	21.4	29.4	166	331	485	617
Percentage sodium	32	42	50	72	74	75	76
Magnesium: calcium ratio	1.06	1.29	1.42	4.33	7.10	5.02	5.18

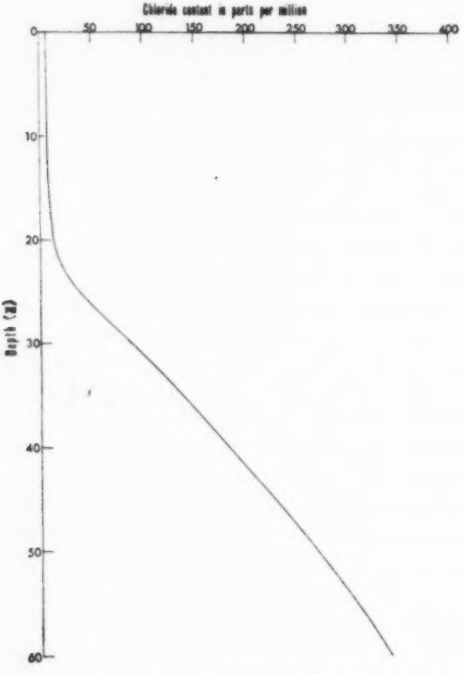

FIG. 1. The increase of chloride ion with depth.

This being the case many marine organisms will be carried into the lake although only those capable of adapting to very low salinities will survive. It is quite possible that this could account for the presence of *M. litoralis* and *G. loricatus* as both animals are capable of withstanding very low salinities or even fresh water.

I am very grateful to Mr J. F. Thomas, head of the Industrial Waters Section of the Canada Department of Mines and Technical Surveys, Ottawa, for carrying out the analyses and for his helpful suggestions. I also thank Mr Miron Olsen of Pacific Western Airlines for his valuable assistance in the sampling.

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Sensitivity of Some Acid-Fast Bacteria of Piscine Origin to Certain Chemotherapeutic Substances

The literature contains few references to work published on the sensitivity of acid-fast organisms isolated from fish to antibiotics and synthetic chemotherapeutic agents used in the treatment of human tuberculosis. The object of this study was therefore to obtain information on this topic with particular reference to strains of acid-fast bacteria isolated from fish in Argentina.

The following five test organisms were used in the experiments: *Mycobacterium anabanti*, isolated from *Macropodus opercularis* by Dr Pierre Besse (Station Centrale d'Hydrobiologie Appliquée, Paris) and received from Dr Ruth Gordon (Rutgers, The State University, New Brunswick, N. J., USA); strains M/A-4 and M/A-5 isolated from *Platypoecilus maculatus* and *Betta splendens*, respectively, in Mar del Plata, Argentina; strains M/A-6 and M/A-7 isolated from *Cynolebias adloffi* and *C. wolterstorfii*, respectively, by Dr R. L. Walford, University of California, Los Angeles, Calif., USA. The bacteria were routinely maintained upon Lowenstein-Jensen slopes, which were incubated at 18–20°C.

The chemotherapeutic substances studied were: isonicotinic acid hydrazide (INAH) ("Nicotibina", Lepetit); *p*-aminosalicylic acid (PAS) (Lab. Warner Argentina); viomycin sulphate ("Viocina", Pfizer); streptomycin sulphate ("Estreptomicina", Lepetit); rifamycin SV ("Rifocina", Lepetit); and kanamycin sulphate (Bristol Laboratories Inc., Syracuse, N.Y., USA). Solutions were prepared at a concentration of 1:250 in sterile distilled water, aseptic precautions being adopted throughout.

Slopes of the synthetic mineral medium of Gordon and Smith (1953) were used for sensitivity tests because previous experience in this laboratory had shown that it supported excellent growth of fish mycobacteria. The formula used was as given by Gordon and Smith in their original publication, but the agar concentration was increased to 25 g/liter to compensate for the addition of aqueous material. The medium was distributed in 3.5-ml quantities and sterilized in the autoclave at 15 lb pressure (121°C) for 15 min. Prior to cooling, each tube received 0.5 ml of sterile 10% aqueous glucose solution, together with 1.0 ml of the appropriate serial dilution of the antibiotic. Tubes were labelled and allowed to solidify in an inclined position. The concentrations of the various antibiotics ranged from 800.0–0.8μg/ml (Johnson *et al.*, 1959).

Emulsions of the test organisms were prepared by washing off the growth from a Lowenstein-Jensen slope and resuspending in sterile distilled water. Following centrifugation and repeated washing, the cells were suspended in distilled water to give an emulsion containing approximately 500,000,000 cells/ml, determining this concentration by reference to a series of nephelometer tubes prepared in this laboratory.

One standard loopful of the test emulsion was streaked onto the surface of each of a series of tubes containing the antibiotics in their respective con-

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centrations, and the tubes were incubated at 22°C. A positive and negative control was set up for each therapeutic agent and readings were taken at 7, 14, and 28 days. The readings were based upon the appearance of growth and on a colour change in the medium indicating that acid had been formed from the glucose.

The results of the experiments are given in Table I. The test organisms show a varying degree of sensitivity to the antibiotics tested, and many of the strains were highly resistant to INAH, PAS, and streptomycin, a finding which is similar to that of Sato (1962) with reference to mycobacteria isolated from species of tropical fish. Strain M/A-5 appears to be quite resistant to all of the substances tested. This organism was isolated in Argentina and therefore it does not seem possible that it could have been exposed previously to any antibiotic substance which would permit the development of resistant strains. It might finally be mentioned that taking into account the action of kanamycin sulphate upon all of the test strains, it has been possible to treat *in vivo* cases of spontaneous piscine tuberculosis caused by strains M/A-4 and M/A-5 by the oral application of this antibiotic. This work will form the basis of a future communication.

TABLE I. Minimum inhibitory concentration (in μg/ml) of the antibiotic at which no growth was observed.

	Organism				
Antibiotic	*Mycobacterium anabanti*	M/A-4	M/A-5	M/A-6	M/A-7
INAH	800[a]	50	800[a]	3.1	50.0
PAS	800[a]	6.3	800[a]	25.0	50.0
Viomycin sulphate	400	12.5	800[a]	1.6	50.0
Rifamycin SV	25.0	1.6	200	3.1	12.5
Streptomycin sulphate	200	12.5	800	6.3	12.5
Kanamycin sulphate	3.1	1.6	100	1.6	6.3

[a]Growth appeared at 800 μg, the highest concentration used.

This work was carried out during the tenure of a contract by the senior author with the Consejo Nacional de Investigaciones Cientificas y Técnicas (Argentina). The second author is grateful to Prof Dra Teresa Satriano de Daurat for leave of absence to collaborate in this work, and also to the Director

of the Institute of Marine Biology, Mar del Plata, for permission to work therein.

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Modification of the Whirling Vessel for Fecundity Studies

In a previous paper on fecundity of the American plaice (Pitt, 1964) the subsampling of eggs with the whirling vessel was described. This apparatus was originally designed by E. Lea and G. Rollefsen of the Fisheries Directorate, Bergen, as a means of subsampling plankton samples, and its structure and operations were described by Wiborg (1951).

From Fig. 1 it can be seen that the movable disk (D) fits snugly against the outlets of the tubes of the sector compartments (Tb) except in the case of the openings where the sample tubes (ST) are in position. This is a metal-against-metal fit and the author has experienced difficulty in preventing leakage if the disk was frequently moved. Hence the latter was allowed to remain in a fixed position leaving only two compartments available for subsampling.

When the openings of the sample tubes (ST) are in position the volume of the sector compartments is increased by the space in the sample tube from the level of the movable disk to a point where the clamp (S) closes the bottom of these tubes. This gives an additional volume of about 0.8 cc. This could accommodate a considerable number of plaice eggs or the eggs of most gadoids, since the former measure 0.7–0.8 mm in diameter (from 10% formalin) and cod and

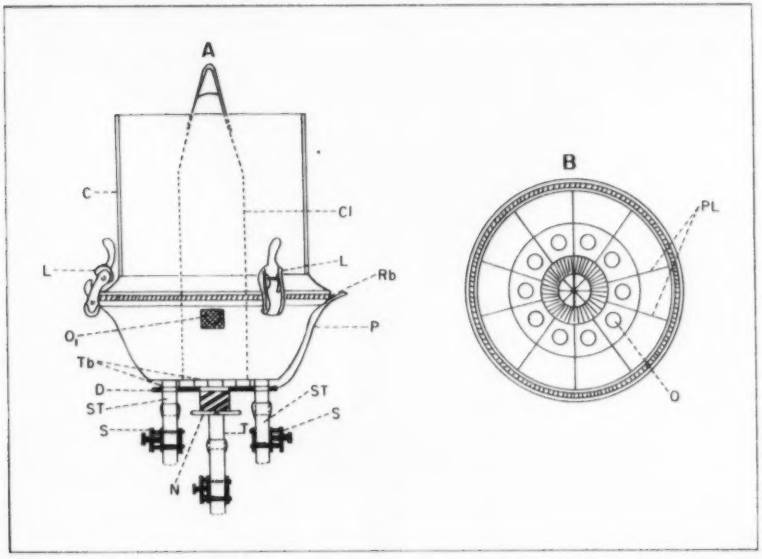

FIG. 1. A, vertical and B, transverse aspect of the whirling vessel (Wiborg, 1951). (C, outer cylinder; Cl, inner cylinder; L, lock; Rb, rubber ring; P, lever; O, opening in inner cylinder, covered by plankton silk; Tb, tubes of the sector compartments; D, movable disk; N, nut; T, central tube; S, clamp; ST, sample tubes; Pl, radial walls.)

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haddock, for example, are smaller than this. This refers to opaque eggs from ovaries as used for fecundity studies.

The possibility of eggs being trapped in the above-mentioned space during the operation of the vessel was investigated. It was found that when 540 eggs were placed in this space and the vessel rotated in the usual way, 310 eggs or 57% were still in the same compartment with the remainder scattered randomly among the other nine.

It thus seems apparent that the rotation of the vessel is insufficient to move the eggs from this confined space. When the vessel is operated correctly, and rotated before the eggs settle, it seems doubtful if many eggs could be trapped in this space. Tests of the equipment by Wiborg (1951) with plankton samples, and Pitt (1964) with plaice, gave good results when the vessel was operated in this way.

Eggs used in fecundity studies are taken before the eggs become translucent and thus have a higher specific gravity than eggs from plankton collections. Therefore in using the whirling vessel in fecundity studies, especially when it is being used by a single operator, there is always the danger that the eggs will settle before it can be rotated.

Table I gives the results of a test of the whirling vessel in which the eggs were allowed to settle before it was rotated. A total of 30 subsamples was

TABLE I. Test of original whirling vessel. Eggs allowed to settle before whirling. Sampling from two compartments only for each operation.

740	751
1032	848
1144	847
929	868
814	849
997	829
879	886
836	900
902	909
834	860
896	858
941	895
862	829
884	873
838	897

$N = 30$; $\bar{X} = 880.9$; $S_x = 76.8$; $S_{\bar{x}} = 14.0$; actual number in sample 7013.

FIG. 2. Photograph of the modified whirling vessel.

TABLE II. A. Test of the modified whirling vessel using a total of 5000 eggs. B. Test of the modified whirling vessel using a whole ovary fractioned to 1/500.

	Compartments											
	1	2	3	4	5	6	7	8	9	10	Total	Mean
A												
1. Eggs settled before rotation	512	563	561	499	417	516	451	577	407	456	4959	495.9
2. " " " "	467	510	421	538	551	394	475	465	576	524	4921	492.1
3. " " " "	573	466	390	553	457	415	567	461	475	476	4833	483.3
4. Vessel rotated while eggs were added	408	463	489	507	553	563	487	487	484	439	4880	488.0
5. All eggs put in one compartment	498	490	515	462	554	461	560	402	435	475	4852	485.2
6. Average (1–5)	491.6	498.4	475.2	511.8	506.4	469.8	508.0	478.4	475.4	474.0		488.9
B												
1. Eggs settled before rotation	600	618	644	649	598	580	591	666	627	613	6186	618.6
2. " " " "	629	632	677	681	593	564	618	617	688	694	6393	639.3

taken, i.e. 15 operations (two sampling compartments only were used). After each subsample was counted it was added to the remaining eggs for the next operation.

The results give an obvious error (Table I). For the 30 subsamples the mean count was 880.9 with the counts ranging from 740 to 1144. The actual number counted at the end of the experiment was 7013 to give a theoretical subsample average of 701.3 for each compartment. The counts are all higher than the expected mean with the latter not even within minus 2 standard deviations of the observed mean. The conclusion was made that eggs being held in the openings of the sample tubes (ST, Fig. 1) led to this error in estimation.

In order to eliminate this possibility and to make the apparatus more accurate under any operating conditions, the movable disk with the sample tubes attached was discarded and the tubes of the sector compartments (Tb, Fig. 1) were replaced with slightly heavier gauge brass sleeves which were threaded internally and fitted with car radiator drain cocks (needle seat drain cocks), the upper ends of which were flush with the bottom of the sector compartments. The modified vessel is illustrated in Fig. 2. The openings of the drain cocks are less than 3 mm in diameter, hence this modification of the whirling vessel would be of little use as a plankton subsampler.

With the modifications of the vessel all 10 compartments are available for sampling and a complete "run through" can be accomplished in about 30 min. Table II gives the results of a series of counts for the modified vessel. From these it can be seen that while there is variation between compartments, for all the tests no single compartment is consistently higher or lower than the others.

The variations between compartments would seem to indicate that a minimum of two compartments should be averaged for each fecundity estimate.

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